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1. Your reference — P104199GB

2. Patent application number *(The Patent Office will fill in this part)* — 0328048.4

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

University of Sheffield
Western Bank
Sheffield
S10 2TN
GB

Patents ADP number *(if you know it)* — 7397683001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — Gene Screen

5. Name of your agent *(if you have one)* — Harrison Goddard Foote

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

31 St Saviourgate
YORK
YO1 8NQ

Patents ADP number *(if you know it)* — 07914237002

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Description	299 (tables 1+2 added to description)
Claim *(s)*	7
Abstract	1
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Date 3/12/03

12. Name and daytime telephone number of person to contact in the United Kingdom — Rob Docherty — 01904 732120

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DUPLICATE

Gene Screen

The invention relates to a screen for the identification of genes which show regulated expression in response to carbon source utilisation.

Colorectal cancer is a cancer which occurs in the large intestine and rectum. The colon can be divided into effectively four sections; the ascending colon; the transverse colon; the descending colon; and the sigmoid colon. Most colorectal cancers arise in the sigmoid colon and develop from "polyps" which can grow for several years before becoming cancerous. The early detection of these pre-cancerous growths is obviously desirable since removal of the polyps is a very effective means to stem the progress of disease.

There are various types of colorectal cancer. Most cancers of this type are adenocarcinomas which are malignant growths which begin in the epithelial cells which line the colon and rectum. Other cancers of the colon and rectum include gastrointestinal stromal tumours and lymphomas. In some examples the patient can be asymptomatic and for this reason it is important that screening is undertaken to identify those patients in which pre-cancerous polyps are forming. However, some patients do present with symptoms and these include rectal bleeding, diarrhoea, constipation, abdominal pain, and general weakness.

As mentioned above, regular screening is by far the most effective way of controlling this disease since removal of pre-cancerous polyps by surgery can effectively cure any disease before it is initiated. Currently, diagnostic tests include the use of colonoscopy, which allows a doctor to examine the rectum and colon; faecal blood analysis to check for any bleeding from the bowel and rectal area although this test is not directly diagnostic for cancerous lesion in its own right; and sigmoidoscopy which is similar to colonoscopy but only investigates the lower bowel area. Typically, patients with a family history of colorectal cancer can be expected to have

a colonoscopy every 5 years or so and a blood stool check on a yearly basis from about the age of 40.

The treatment of colorectal cancer usually involves invasive surgery to remove polyps and/or malignant growths. If the cancer has developed beyond the polyp stage then more extensive surgery is required which can result in removal of part of the bowel and surrounding lymph nodes. In the situation where a cancer necessitates extensive surgery a colostomy stoma may be required, at least for a period, to allow the bowel to recover from surgery. Surgery in the rectal region is more complicated and is largely dependent on how far the disease has progressed. In some cases the surgery can damage nerves which control sexual and urinary functions. In advanced stage colorectal cancers metastatic lesions may require removal and in about 15% of cases the lesions are in the liver which requires removal of large parts of the liver. The surgical removal of polyps and/or cancerous growths lead to a good prognosis for patients. In some cases surgery is followed by a course of chemotherapy (for colon cancer) and chemotherapy and radiation therapy (rectal cancer) to remove any cancer cells not detected during surgery. The chemotherapeutic agents typically used to treat colorectal cancer include 5-fluorouracil, leucovorin, irinotecan and capecitabine.

It is apparent that the early detection of cells which are pre-cancerous is highly desirable since in most cases surgery to remove these cells results in a very good prognosis for patients. Diagnostic tests which use the detection of cancer markers as an early indicator of cancer are known in the art.

For example, EP1355149 describes gene expression profiles from colorectal samples to provide a "finger print" expression profile as an indication of whether a patient is susceptible to the development of colorectal cancer or indeed if malignant growth has already been initiated. The disclosure in EP1355149 is directed to the use of microarrays to compare transformed and non-transformed tissue gene expression in a global sense.

WO02/059609 also describes a gene screen which utilises expression profiles in breast and colorectal cancer. A comparison is made between "normal" and "abnormal" samples in patients to provide a global picture of gene expression in these samples as an indicator of particular genes which are either over-expressed or abrogated between samples. Both EP1355149 and WO02/059609 take a shot gun approach to screening for target genes which can be used either as a diagnostic tool or as a target for the development of new chemotherapeutic agents.

The present invention provides a targeted screen for genes the expression of which may be altered in a response to carbon source. The invention makes use of the differences in expression profiles between normal and diseased tissue as a consequence of differences in metabolic state between cancer cells and normal cells due in part to carbon source utilisation by these respective cell types. The epithelial cells which line the colon and rectum metabolise butyrate as a carbon source for energy transduction via glycolysis. The main carbon source utilised by tumour cells is glucose. Consequently, expression profiles between these cell types are different due to the differences in carbon source metabolism.

We have identified a large number of potential markers of colorectal cancer which have utility with respect to the early diagnosis of disease and as targets for the development of novel chemotherapeutic agents. Moreover, this assay has broader applicability to conditions resulting from dysfunction of the bowel (e.g colitis, ulcerative colitis, diversion colitis. Crohn's disease and irritable bowel syndrome. In addition the assay provides a screening tool for fibre consumption and as an assay for colon microflora functionality (the effectiveness of fermentation of specific fibres) .

According to an aspect of the invention there is provided a method to screen for nucleic acid molecules which show altered expression in an isolated first cell sample comprising comparing the gene expression profiles between said first cell sample with a second reference cell sample wherein said first cell sample has been grown in

the presence of the carbon source butyrate, or a related carbon source from which butyrate is derived, either directly or indirectly, and comparing said expression profile with the expression profile in said second reference cell sample which has not been grown in the presence of butyrate, or said related carbon source.

According to a further aspect of the invention there is provided a method to screen for nucleic acid molecules which show altered expression in an isolated biological sample comprising the steps of:

i) providing
   a) a cell growth preparation comprising a first cell sample derived from at least one region of the colon; cell growth media; and a carbon source wherein said carbon source is butyrate; and
   b) a cell growth preparation comprising a second cell sample derived from an equivalent region of the colon; cell growth media; and a carbon source which is not butyrate;

ii) extracting nucleic acid from said first and second cell samples; and

iii) comparing the gene expression profile in said first cell sample with the gene expression profile in said second cell sample.

In a preferred method of the invention said first and second cell samples are derived from the ascending colon.

In an alternative preferred method of the invention said first and second cell samples are derived from the transverse colon.

In a further preferred method of the invention said first and second samples are derived from the descending colon.

In a still further preferred method of the invention said first and second samples are derived from the sigmoid region of the colon. Preferably said cell samples are derived from the rectal region of the colon.

In a further preferred method of the invention said first and second cell samples comprise epithelial cells.

In a preferred method of the invention said carbon source which is not butyrate is glucose.

In a still further preferred method of the invention said nucleic acid molecule which shows altered expression is selected from the group as represented by the nucleic acid sequences shown in Table 1, or nucleic acid molecules which hybridise to the sequences presented Table 1. Preferably said nucleic acid molecules hybridise under stringent hybridisation conditions.

According to a further aspect of the invention there is provided a method for the detection of at least one nucleic acid molecule associated with the initiation and/or progression of colorectal cancer, in an animal, comprising the steps of:

i) providing a biological sample comprising at least one cell to be tested;

ii) contacting said sample with a ligand which binds at least one nucleic acid molecule as represented by the nucleic acid sequence selected from the group consisting of:

    a) a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1;

    b) a nucleic acid molecule which hybridises to nucleic acid molecules as defined in (a);

    c) a nucleic acid molecule that is degenerate as a consequence of the genetic code to the nucleic acid molecule represented in (a) and (b);

iii) detecting the presence of at least one nucleic acid molecule in said sample.

In a preferred method of the invention said animal is human.

In a further preferred method of the invention said colorectal cancer is adenocarcinoma.

In a preferred method of the invention said ligand is a nucleic acid molecule adapted to anneal to said nucleic acid molecule which is indicative of colorectal cancer.

It will be apparent to the skilled person that a number of nucleic acid based assay systems are available which can be adapted to detect nucleic acid molecules as hereindisclosed. For example quantitative polymerase chain reaction assays, *in situ* hybridisation, northern blot.

According to a further aspect of the invention there is provided a method for the detection of at least one polypeptide associated with the initiation and/or progression of colorectal cancer, in an animal, comprising the steps of:

i) providing a biological sample comprising at least one cell to be tested;

ii) contacting said sample with at least one ligand which ligand specifically binds at least one polypeptide encoded by a nucleic acid molecule as represented by the nucleic acid sequence shown in Table 1, or a variant polypeptide comprising an amino acid sequence which varies by the addition, deletion or substitution of at least one amino acid residue; and

iii) detecting the presence of at least one polypeptide in said sample.

In a preferred method of the invention said animal is human.

In a further preferred embodiment of the invention said ligand is an antibody, preferably a monoclonal antibody, or at least the effective binding part thereof.

Methods which utilise antibodies to detect the presence of a polypeptide in a biological sample are well known in the art and include ELISA's, western blot and immunofluoresence.

According to a further aspect of the invention there is provided the use of at least one polypeptide, or variant sequence thereof, encoded by a nucleic acid molecule(s) as represented by the nucleic acid sequences as shown in Table 1, as a target for the screening of agents which modulate the activity of said polypeptide.

According to a yet further aspect of the invention there is provided a method to screen for agents which modulate the activity of at least one gene associated with the initiation and/or progression of colorectal cancer comprising the steps of:

i) forming a preparation comprising at least one polypeptide wherein said polypeptide is encoded by a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1, or a variant polypeptide comprising an amino acid sequence which varies by the addition, deletion or substitution of at least one amino acid residue as represented by the amino acid sequences shown in Table 1, and at least one agent to be tested; and

ii) determining the activity of said agent with respect to activity of said polypeptide.

In a preferred method of the invention said polypeptide is expressed by a cell wherein said cell is transformed or transfected with said nucleic acid molecule. Preferably said nucleic acid molecule is part of a vector adapted for recombinant expression of said nucleic acid molecule. Preferably said vector is provided with a promoter which enables the expression of said nucleic acid molecule to be regulated.

In a preferred method of the invention said cell is derived from the colon, preferably said cell is an epithelial cell which lines said colon.

In a further preferred method of the invention said agent is an antibody, preferably a monoclonal antibody or modified antibody, or at least the effective binding part thereof.

Antibodies, also known as immunoglobulins, are protein molecules which usually have specificity for foreign molecules (antigens). Immunoglobulins (Ig) are a class of structurally related proteins consisting of two pairs of polypeptide chains, one pair of light (L) (low molecular weight) chain (κ or λ), and one pair of heavy (H) chains (γ, α, μ, δ and ε), all four linked together by disulphide bonds. Both H and L chains have regions that contribute to the binding of antigen and that are highly variable from one Ig molecule to another. In addition, H and L chains contain regions that are non-variable or constant.

The L chains consist of two domains. The carboxy-terminal domain is essentially identical among L chains of a given type and is referred to as the "constant" (C) region. The amino terminal domain varies from L chain to L chain and contributes to the binding site of the antibody. Because of its variability, it is referred to as the "variable" (V) region.

The H chains of Ig molecules are of several classes, α, μ, σ, α, and γ (of which there are several sub-classes). An assembled Ig molecule consisting of one or more units of two identical H and L chains, derives its name from the H chain that it possesses. Thus, there are five Ig isotypes: IgA, IgM, IgD, IgE and IgG (with four sub-classes based on the differences in the 'constant' regions of the H chains, i.e., IgG1, IgG2, IgG3 and IgG4). Further detail regarding antibody structure and their various functions can be found in, Using Antibodies: A laboratory manual, Cold Spring Harbour Laboratory Press.

In a preferred method of the invention said fragment is a Fab fragment.

In a further preferred method of the invention said antibody is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; and antibodies comprising CDR3 regions.

Preferably said fragments are single chain antibody variable regions (scFV's) or domain antibodies. If a hybridoma exists for a specific monoclonal antibody it is well within the knowledge of the skilled person to isolate scFv's from mRNA extracted from said hybridoma via RT PCR. Alternatively, phage display screening can be undertaken to identify clones expressing scFv's. Domain antibodies are the smallest binding part of an antibody (approximately 13kDa). Examples of this technology is disclosed in US6, 248, 516, US6, 291, 158, US6,127, 197 and EP0368684 which are all incorporated by reference in their entirety.

A modified antibody, or variant antibody and reference antibody, may differ in amino acid sequence by one or more substitutions, additions, deletions, truncations which may be present in any combination. Among preferred variants are those that vary from a reference polypeptide by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid by another amino acid of like characteristics. The following non-limiting list of amino acids are considered conservative replacements (similar): a) alanine, serine, and threonine; b) glutamic acid and asparatic acid; c) asparagine and glutamine d) arginine and lysine; e) isoleucine, leucine, methionine and valine and f) phenylalanine, tyrosine and tryptophan. Most highly preferred are variants which show enhanced biological activity.

Preferably said antibody is a humanised or chimeric antibody.

A chimeric antibody is produced by recombinant methods to contain the variable region of an antibody with an invariant or constant region of a human antibody.

A humanised antibody is produced by recombinant methods to combine the complementarity determining regions (CDRs) of an antibody with both the constant (C) regions and the framework regions from the variable (V) regions of a human antibody.

Chimeric antibodies are recombinant antibodies in which all of the V-regions of a mouse or rat antibody are combined with human antibody C-regions. Humanised antibodies are recombinant hybrid antibodies which fuse the complimentarity determining regions from a rodent antibody V-region with the framework regions from the human antibody V-regions. The C-regions from the human antibody are also used. The complimentarity determining regions (CDRs) are the regions within the N-terminal domain of both the heavy and light chain of the antibody to where the majority of the variation of the V-region is restricted. These regions form loops at the surface of the antibody molecule. These loops provide the binding surface between the antibody and antigen.

Antibodies from non-human animals provoke an immune response to the foreign antibody and its removal from the circulation. Both chimeric and humanised antibodies have reduced antigenicity when injected to a human subject because there is a reduced amount of rodent (i.e. foreign) antibody within the recombinant hybrid antibody, while the human antibody regions do not elicit an immune response. This results in a weaker immune response and a decrease in the clearance of the antibody. This is clearly desirable when using therapeutic antibodies in the treatment of human diseases. Humanised antibodies are designed to have less "foreign" antibody regions and are therefore thought to be less immunogenic than chimeric antibodies.

In an alternative preferred method of the invention said agent is a polypeptide or a peptide. Preferably said polypeptide or peptide is modified.

In a preferred method of the invention said peptide is at least 6 amino acid residues in length. Preferaby the length of said peptide/polypeptide is selected from the group

consisting of: at least 7 amino acid residues; 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues in length. Alternatively the length of said peptide/polypeptide is at least 20 amino acid residues; 30; 40; 50; 60; 70; 80; 90; or 100 amino acid residues in length.

It will be apparent to one skilled in the art that modification to the amino acid sequence of peptide agents could enhance the binding and/or stability of the peptide with respect to its target sequence. In addition, modification of the peptide may also increase the *in vivo* stability of the peptide thereby reducing the effective amount of peptide necessary to inhibit the activity of a target polypeptide. This would advantageously reduce undesirable side effects which may result *in vivo*. Alternatively or preferably, said modification includes the use of modified amino acids in the production of recombinant or synthetic forms of peptides. It will be apparent to one skilled in the art that modified amino acids include, by way of example and not by way of limitation, 4-hydroxyproline, 5-hydroxylysine, N^6-acetyllysine, N^6-methyllysine, N^6,N^6-dimethyllysine, N^6,N^6,N^6-trimethyllysine, cyclohexyalanine, D-amino acids, ornithine. Other modifications include amino acids with a C_2, C_3 or C_4 alkyl R group optionally substituted by 1, 2 or 3 substituents selected from halo (e.g. F, Br, I), hydroxy or C_1-C_4 alkoxy. Modifications also include, by example and not by way of limitation, acetylation and amidation.

In a preferred embodiment of the invention said peptide sequence is acetylated. Preferably said acetylation is to the amino terminus of said peptide.

In a further preferred embodiment of the invention said peptide sequence is amidated. Preferably said amidation is to the carboxyl-terminus of said peptide.

It will also be apparent to one skilled in the art that peptides could be modified by cyclisation. Cyclisation is known in the art, (see Scott *et al* Chem Biol (2001), 8:801-815; Gellerman et al J. Peptide Res (2001), 57: 277-291; Dutta *et al* J. Peptide

Res (2000), 8: 398-412; Ngoka and Gross J Amer Soc Mass Spec (1999), 10:360-363.

In a further preferred method of the invention said agent is nucleic acid molecule. Preferably said nucleic acid molecule is an aptamer or a modified aptamer. In an alternative preferred method of the invention said nucleic acid is an inhibitory RNA (RNAi) molecule. Alternatively said nucleic acid molecule is an antisense nucleic acid molecule.

Nucleic acids have both linear sequence structure and a three dimensional structure which in part is determined by the linear sequence and also the environment in which these molecules are located. Conventional therapeutic molecules are small molecules, for example, peptides, polypeptides, or antibodies, which bind target molecules to produce an agonistic or antagonistic effect. It has become apparent that nucleic acid molecules also have potential with respect to providing agents with the requisite binding properties which may have therapeutic utility. These nucleic acid molecules are typically referred to as aptamers. Aptamers are small, usually stablised, nucleic acid molecules which comprise a binding domain for a target molecule. A screening method to identify aptamers is described in US 5,270,163, which is incorporated by reference. Aptamers are typically oligonucleotides which may be single stranded oligodeoxynucleotides, oligoribonucleotides, or modified oligodeoxynucleotide or oligoribonucleotides.

The term "modified" encompasses nucleotides with a covalently modified base and/or sugar. For example, modified nucleotides include nucleotides having sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified nucleotides may also include 2' substituted sugars such as 2'-O-methyl-; 2-O-alkyl; 2-O-allyl; 2'-S-alkyl; 2'-S-allyl; 2'- fluoro-; 2'-halo or 2;azido-ribose, carbocyclic sugar analogues a-anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, and sedoheptulose.

Modified nucleotides are known in the art and include by example and not by way of limitation; alkylated purines and/or pyrimidines; acylated purines and/or pyrimidines; or other heterocycles. These classes of pyrimidines and purines are known in the art and include, pseudoisocytosine; N4, N4-ethanocytosine; 8-hydroxy-N6-methyladenine; 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil; 5-fluorouracil; 5-bromouracil; 5-carboxymethylaminomethyl-2-thiouracil; 5-carboxymethylaminomethyl uracil; dihydrouracil; inosine; N6-isopentyl-adenine; 1-methyladenine; 1-methylpseudouracil; 1-methylguanine; 2,2-dimethylguanine; 2-methyladenine; 2-methylguanine; 3-methylcytosine; 5-methylcytosine; N6-methyladenine; 7-methylguanine; 5- methylaminomethyl uracil; 5-methoxy amino methyl-2-thiouracil; β-D-mannosylqueosine; 5-methoxycarbonylmethyluracil; 5-methoxyuracil; 2 methylthio-N6-isopentenyladenine; uracil-5-oxyacetic acid methyl ester; psueouracil; 2-thiocytosine; 5-methyl-2 thiouracil, 2-thiouracil; 4-thiouracil; 5-methyluracil; N-uracil-5-oxyacetic acid methylester; uracil 5—oxyacetic acid; queosine; 2-thiocytosine; 5-propyluracil; 5-propylcytosine; 5-ethyluracil; 5-ethylcytosine; 5-butyluracil; 5-pentyluracil; 5-pentylcytosine; and 2,6,-diaminopurine; methylpsuedouracil; 1-methylguanine; 1-methylcytosine.

The aptamers of the invention are synthesized using conventional phosphodiester linked nucleotides and synthesized using standard solid or solution phase synthesis techniques which are known in the art. Linkages between nucleotides may use alternative linking molecules. For example, linking groups of the formula P(O)S, (thioate); P(S)S, (dithioate); P(O)NR'2; P(O)R'; P(O)OR6; CO; or CONR'2 wherein R is H (or a salt) or alkyl (1-12C) and R6 is alkyl (1-9C) is joined to adjacent nucleotides through –O- or –S-. The binding of aptamers to a target polypeptide is readily testable.

An alternative nucleic acid molecule is a so called RNAi molecule. A recent technique to specifically ablate gene function is through the introduction of double stranded RNA, also referred to as inhibitory RNA (RNAi), into a cell which results

in the destruction of mRNA complementary to the sequence included in the RNAi molecule. The RNAi molecule comprises two complementary strands of RNA (a sense strand and an antisense strand) annealed to each other to form a double stranded RNA molecule. The RNAi molecule is typically derived from exonic or coding sequence of the gene which is to be ablated. Recent studies suggest that RNAi molecules ranging from 100-1000bp derived from coding sequence are effective inhibitors of gene expression. Surprisingly, only a few molecules of RNAi are required to block gene expression which implies the mechanism is catalytic. The site of action appears to be nuclear as little if any RNAi is detectable in the cytoplasm of cells indicating that RNAi exerts its effect during mRNA synthesis or processing.

In a preferred method of the invention there is provided a cassette comprising a nucleic acid molecule, or part thereof, wherein said molecule is selected from the group consisting of:

i) a nucleic acid molecule represented by the nucleic acid sequence shown in Table 1 ;

ii) a nucleic acid molecule which hybridises to the sequence in (i) above and which encodes a polypeptide which initiates or promotes transformation of colon cells; or

iii) a nucleic acid molecule which is degenerate because of the genetic code to the sequences defined in (i) and (ii) above, wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

In a preferred method of the invention said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

In a further preferred method of the invention said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their

sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

In a preferred embodiment of the invention said first and second parts are linked by at least one nucleotide base.

In a preferred embodiment of the invention said first and second parts are linked by 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 nucleotide bases.

In a further preferred embodiment of the invention the length of the RNAi molecule is between 100bp-1000bp. More preferably still the length of RNAi is selected from 100bp; 200bp; 300bp; 400bp; 500bp; 600bp; 700bp; 800bp; 900bp; or 1000bp. More preferably still said RNAi is at least 1000bp.

In an alternative preferred method of the invention the RNAi molecule is between 15bp and 25bp, preferably said molecule is 21bp. Preferably said cassette is part of a vector.

According to a further aspect of the invention there is provided an antibody identified by the method according to the invention for use as a pharmaceutical.

According to a further aspect of the invention there is provided a polypeptide or peptide identified by the method according to the invention for use as a pharmaceutical.

According to a further aspect of the invention there is provided a nucleic acid molecule identified by the method according to the invention for use as a pharmaceutical.

In a preferred embodiment of the invention said nucleic acid molecule is an aptamer.

In an alternative preferred embodiment of the invention said nucleic acid molecule is an inhibitory RNA.

In a further alternative preferred embodiment of the invention said nucleic acid molecule is an antisense nucleic acid molecule.

In a preferred embodiment of the invention said pharmaceutical further comprises a a diluent, carrier or excipient.

When administered, the therapeutic compositions of the present invention are administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents, such as chemotherapeutic agents.

The therapeutics of the invention can be administered by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal. When antibodies are used therapeutically, a preferred route of administration is by pulmonary aerosol. Techniques for preparing aerosol delivery systems containing antibodies are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the antibodies, such as the paratope binding capacity (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing antibody aerosols without resort to undue experimentation. When using antisense preparations of the invention, slow intravenous administration is preferred.

The compositions of the invention are administered in effective amounts. An "effective amount" is that amount of a composition that alone, or together with further doses, produces the desired response. In the case of treating a particular disease, such as cancer, the desired response is inhibiting the progression of the disease. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine methods or can be monitored according to diagnostic methods of the invention discussed herein.

Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

The pharmaceutical compositions used in the foregoing methods preferably are sterile and contain an effective amount for producing the desired response in a unit of weight or volume suitable for administration to a patient. The response can, for example, be determined by measuring the physiological effects of the composition, such as regression of a tumour, decrease of disease symptoms, modulation of apoptosis, etc.

The doses of pharmaceutical agent administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of

administration used and the state of the subject. Other factors include the desired period of treatment. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits.

In general, doses of pharmaceutical are formulated and administered in doses between 1 ng and about 500mg, and between 10 ng and 100mg, according to any standard procedure in the art. Where nucleic acids are employed, doses of between 1 ng and 0.1mg generally will be formulated and administered according to standard procedures. Other protocols for the administration of compositions will be known to one of ordinary skill in the art, in which the dose amount, schedule of injections, sites of injections, mode of administration (e.g., intra-tumoral) and the like vary from the foregoing. Administration of pharmaceutical compositions to mammals other than humans, e.g. for testing purposes or veterinary therapeutic purposes, is carried out under substantially the same conditions as described above. A subject, as used herein, is a mammal, preferably a human, and including a non-human primate, cow, horse, pig, sheep, goat, dog, cat or rodent.

When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic,

malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

Pharmaceutcial compositions may be combined, if desired, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt.

The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous or non-aqueous preparation of pharmaceutical agents, which is preferably isotonic with the blood of the recipient. This preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

An embodiment of the invention will now be described by example only and with reference to the following Figures and Tables;

Figure 1 illustrates a concentration-response of cells growing in butyrate as sole carbon source. This is the summary of four independent repeat experiments. Legend shows butyrate concentrations in mM;

Figure 2 illustrates the purity and quality of RNA preparation. The 28S and 18S sample bands are tight and clearly resolved for RNA prepared from butyrate- and glucose-grown cells. Little or no DNA or salt contamination appears in the samples;

Table1 illustrates nucleic acid and protein sequences identified by the screening method according to the invention; and

Table 2 illustrates a summary of expression data of nucleic acid sequences identified in Table 1.

Materials and Methods

We have compared the expression profiles of colon cells growing in either glucose or butyrate as a carbon source. HT 29 colon carcinoma cells were cultured in DMEM medium (Gibco) in the presence of 10% foetal calf serum, penicillin and streptomycin. Cells were either cultured in glucose alone as the sole carbon source, or in butyrate as the sole extraneous provided carbon source. Empirical analysis of HT29 cells grown in multiple butyrate concentrations revealed that 2mM butyrate was optimal for cell culture in the absence of glucose. Cells were cultured in either medium for multiple passages (typically 4). RNA was extracted from cells grown in each condition and used to probe an Affymetrix human 12k array. The expression profile of cells cultured in each condition was compared and genes altered in expression by more than 2 fold are listed in Table 2.

Materials used during this study

ITEM	ITEM - SPECIFICS	SUPPLIER
Glucose medium (1)	Dulbecco's Modified Eagle Medium 25 mM HEPES 1 x 0.1 micron filtered with sodium pyruvate, with 1000	GIBCO

	mg/l glucose with pyridoxine + FCS + p/s (500 ml)	
Butyrate medium (2) 0.2 mM NaB medium	Dulbecco's Modified Eagle Medium 1 x 0.1 micron filtered with L-glutamine without glucose, without sodium pyruvate + NaB (1M) 110 μl + FCS + p/s (555.1 ml)	GIBCO
Butyrate medium (3) 2 mM NaB medium	Dulbecco's Modified Eagle Medium 1 x 0.1 micron filtered with L-glutamine without glucose, without sodium pyruvate + NaB (1M) 1100 μl + FCS + p/s (556.1 ml)	GIBCO
Medium without glucose and without butyrate (4)	Dulbecco's Modified Eagle Medium 1 x 0.1 micron filtered with L-glutamine without glucose, without sodium pyruvate + FCS + p/s (550 ml)	GIBCO
NaB stock	Sodium Butyrate powder dissolved in sterile water 250 mg in 2.27 ml water	Sigma

	(1M) 0.2 μm filter sterilised	
Sterile syringes	5 ml	Becton Dickinson UK, Ltd
Sterilising filters	0.2 μm Acrodisc	Gelman Sciences, Ltd
Item	**Item specifics**	**Supplier**
FCS	Foetal Calf Serum 50 ml per 500 ml DMEM	Harlan Sera Lab
P/S	Penicillin – Streptomycin solution 100ml bottle (100 X) – 5 ml per 500 ml DMEM	Sigma
TE for splitting cells	Trypsin Enzyme – 100 ml bottle - 3 ml per T75 and 1 ml per 6 well plate well	Sigma
FCS tubes	50 ml Centrifuge tubes	Corning Inc
P/S + TE tubes	30 ml Universal containers	Bibby Sterilin Ltd
Tissue Culture Plates	6 well sterile with lid single packed	Greiner bio-one
Tissue Culture Flasks	T 75	Nunclon
Stripette ® 5ml, 10ml,	Serological Pipette,	Corning Inc / Costar

25 ml	individually wrapped	
Pipette	Powerpette plus	Jencons
Cell Counting Slide	Haemocytometer, improved Neubauer	Neubauer
Ethanol for tissue culture	70 % EtOH	Sigma
Virkon for cell culture	1 % Virkon	Day Impex, Ltd
Microscope for cell work	Light 6 – 10X	CK Olympus, Tokyo
Paper towels	Blue	Jamont (UK), Ltd
Latex-free examination gloves	Large	Shermond Surgical Supply, Ltd
Item	**Item specifics**	**Supplier**
RNA extraction reagent	TRIzol ® Reagent	Invitrogen – Life technologies
RNA extraction reagent	Chloroform	Sigma
RNA extraction reagent	Isopropyl alcohol	Sigma

RNA extraction reagent	75% EtOH in DEPC-treated water	Sigma
RNA extraction reagent	Rnase-free water	Sigma
RNA clean up kit	Rneasy Midi Kit (10 RNeasy midi spin columns)	Qiagen
β- Mercaptoethanol	14.3 M stock solution	Sigma
Ethanol for Qiagen	96-100% EtOH	Sigma
Agarose	1g in 100 ml TB-EDTA-Buffer	Helena Biosciences, UK
TB-EDTA- Buffer	Tris-Borate-EDTA buffer 100ml	Sigma
Eppendorf tubes	1.5 ml	Sarstedt Laboratory supplies, Ltd
Loading buffer	6 X	Promega

The Human Colon Carcinoma Cell Line - HT29

The HT29 cell line is established from a colon adenocarcinoma which was removed from a 44 year old Caucasian woman. The cell line is epithelial in origin and hypertriploid. It has been shown to be tumourigenic in nude mice and synthesizes Carcino embryonic antigen - CEA (Egan & Todd, 1972) and the Transforming

growth factors - TGF-α and TGF-β (Anzano *et al.* 1989) when maintained *in vitro*. The HT29 cell line constitutively over-produces mutant p53 protein as a consequence of a point mutation at codon 273, resulting in an Arginine to Histidine amino acid substitution (Hsu *et al.* 1994).

The Culture of HT29 Colorectal adenocarcinoma cells

Cells were cultured in T75 tissue culture flasks (Nunclon) in 5% CO_2 at 37°C. Cells were passaged when confluent by washing twice in PBS and incubating in pre-warmed trypsin : EDTA (1:1) at 37°C until cells detached. The cells were then re-suspended in the appropriate growth medium, either glucose DMEM or butyrate DMEM before being seeded into new T75 tissue culture flasks or 6-well plates.

Optimisation of HT29 cell growth in butyrate as sole extraneous carbon source

HT29 cells were seeded out into 19 wells (in 6 well plates) at a cell density of 0.5 x 10^6 cells per well (i.e. 500 000 cells per well) deduced with the aid of a Haemocytometer (Improved Neubauer). These cells were taken from T75 - 0.2 mM butyrate (NaB) DMEM flasks and allowed to adhere to the 6-well plates over 72 hrs also in 0.2 mM NaB DMEM with FCS and Penicillin / Streptomycin antibiotics. After the cells had adhered to the surface of the 6 well plates the 0.2 mM NaB DMEM was removed and each well was washed twice with PBS in order to remove all traces of the 0.2 mM DMEM, then different concentrations of NaB DMEM with FCS and with Penicillin / Streptomycin antibiotics were added to the appropriate wells in triplicate. Cell counts were taken at various time points. Specific media was changed daily in order to maintain the appropriate / desired NaB concentrations per well. All solutions / reagents used were pre-warmed in a water bath prior to use so as to avoid any cold shock to the cells.

RNA extraction using TRIzol® Reagent

Total RNA was extracted from HT29 cells grown to confluence in T75 flasks using TRIzol Reagent as per manufacturer's recommendations. Cells were grown for several passages either in butyrate-containing medium, or in glucose-containing medium prior to extraction of RNA

Cells were homogenised using 1 ml TRIzol Reagent per 10 cm^2 area of culture surface. The homogenised samples were incubated for 5 minutes at at ambient temperature to permit the complete dissociation of nucleoprotein complexes. 200µl of chloroform was added to each sample. Tubes were shaken vigorously by hand for 15 seconds and incubated at ambient temperature for 3 minutes. Samples were centrifuged at 12000g for 15 minutes at 4oC. RNA in the aqueous phase was separated and precipitated using isopropyl alcohol. RNA was rinsed, air dried and redissolved in RNase-free water.

RNA was further purified using Qiagen RNeasy columns. The columns were used exactly as per manufacturers recommendations. RNA was eluted into RNase-free water.

RNA purified in this way was analysed by agarose gel to establish purity and quality. The gel is shown in figure 2.

Microarray analysis

Microarray analysis was undertaken as a commercial service by the University of Newcastle-upon-Tyne. In this study, the 2 RNA samples (1x butyrate + 1x glucose) from the 2 experimental conditions (butyrate + glucose) were sent to the Institute for Human Genetics at the University of Newcastle-upon-Tyne for microarray analysis. This was performed on a 12 k Affymetrix *Homo sapiens* gene chip. Genes altered in expression by more than 2 fold on the microarray are listed in table 1.

Claims

1. A method to screen for nucleic acid molecules which show altered expression in an isolated first cell sample comprising comparing the gene expression profiles between said first cell sample with a second reference cell sample wherein said first cell sample has been grown in the presence of the carbon source butyrate, or a related carbon source from which butyrate is derived, either directly or indirectly, and comparing said expression profile with the expression profile in said second reference cell sample which has not been grown in the presence of butyrate, or said related carbon source.

2. A method according to Claim 1 wherein said screen for nucleic acid molecules comprises the steps of:

i) providing

a) a cell growth preparation comprising a first cell sample derived from at least one region of the colon; cell growth media; and a carbon source wherein said carbon source is butyrate; and

b) a cell growth preparation comprising a second cell sample derived from an equivalent region of the colon; cell growth media; and a carbon source which is not butyrate;

ii) extracting nucleic acid from said first and second cell samples; and

iii) comparing the gene expression profile in said first cell sample with the gene expression profile in said second cell sample.

3. A method according to Claim 1 or 2 wherein said first and second cell samples are derived from the ascending colon.

4. A method according to Claim 1 or 2 wherein said first and second cell samples are derived from the transverse colon.

5. A method according to Claim 1 or 2 wherein said first and second samples are derived from the descending colon.

6. A method according to Claim 1 or 2 wherein said first and second samples are derived from the sigmoid region of the colon.

7. A method according to Claim 6 wherein said cell samples are derived from the rectal region of the colon.

8. A method according to any of Claims 1-7 wherein said first and second cell samples comprise epithelial cells.

9. A method according to any of Claims 1-8 wherein said carbon source which is not butyrate is glucose.

10. A method according to any of Claims 1-9 wherein said nucleic acid molecule which shows altered expression is selected from the group as represented by the nucleic acid sequences as shown in Table 1, or nucleic acid molecules which hybridise to the sequences presented in Table 1.

11. A method for the detection of at least one nucleic acid molecule associated with the initiation and/or progression of colorectal cancer, in an animal, comprising the steps of:

i) providing a biological sample comprising at least one cell to be tested;

ii) contacting said sample with a ligand which binds at least one nucleic acid molecule as represented by the nucleic acid sequence selected from the group consisting of:

a) a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1;

b) a nucleic acid molecule which hybridises to nucleic acid molecules as defined in (a);

c) a nucleic acid molecule that is degenerate because of the genetic code to the nucleic acid molecule represented in (a) and (b); and

iii) detecting the presence of at least one nucleic acid molecule in said sample.

12. A method according to Claim 11 wherein said colorectal cancer is adenocarcinoma.

13. A method according to Claim 11 or 12 wherein said ligand is a nucleic acid molecule adapted to anneal to said nucleic acid molecule which is associated with colorectal cancer.

14. A method according to Claim 13 wherein said method is a polymerase chain reaction method.

15. A method for the detection of at least one polypeptide associated with the initiation and/or progression of colorectal cancer, in an animal, comprising the steps of:

i) providing a biological sample comprising at least one cell to be tested;

ii) contacting said sample with at least one ligand which ligand specifically binds at least one polypeptide encoded by a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1, or a variant polypeptide comprising an amino acid sequence which varies by the addition, deletion or substitution of at least one amino acid residue of the amino acid sequence shown in Table 1; and

iii) detecting the presence of at least one polypeptide in said sample.

16 A method according to any of Claims 11-15 wherein said animal is human.

17. A method according to Claim 15 or 16 wherein said ligand is an antibody.

18. A method according to Claim 17 wherein said antibody is a monoclonal antibody, or at least the effective binding part thereof.

19. The use of at least one polypeptide, or variant sequence thereof, encoded by a nucleic acid molecule(s) as represented by the nucleic acid sequence as shown in Table 1, as a target for the screening of agents which modulate the activity of said polypeptide.

20. A method to screen for agents which modulate the activity of at least one polypeptide encoded by a gene associated with the initiation and/or progression of colorectal cancer comprising the steps of:

i) forming a preparation comprising at least one polypeptide wherein said polypeptide is encoded by a nucleic acid sequence as shown in Table 1, or a variant polypeptide comprising an amino acid sequence which varies by the addition, deletion or substitution of at least one amino acid residue of the amino acid sequence shown in Table 1 and at least one agent to be tested; and

ii) determining the activity of said agent with respect to activity of said polypeptide.

21. A method according to Claim 20 wherein said polypeptide is expressed by a cell wherein said cell is transformed or transfected with said nucleic acid molecule.

22. A method according to Claim 21 wherein said nucleic acid molecule is part of a vector adapted for recombinant expression of said nucleic acid molecule.

23. A method according to Claim 22 wherein said vector is provided with a promoter which enables the expression of said nucleic acid molecule to be regulated.

24. A method according to any of Claims 21-23 wherein said cell is derived from the colon.

25. A method according to Claim 24 wherein said cell is an epithelial cell.

26. A method according to any of Claims 20-25 wherein said agent is an antibody.

27. A method according to Claim 26 wherein said antibody is a monoclonal antibody or modified monoclonal antibody, or at least the effective binding part thereof.

28. A method according to Claim 27 wherein said binding part is a Fab fragment.

29. A method according to Claim 28 wherein said antibody is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; antibodies comprising CDR3 regions, and single chain antibody variable regions.

30. A method according to Claim 26 wherein said antibody is a humanised.

31. A method according to Claim 26 wherein said antibody is a chimeric antibody.

32. A method according to any of Claims 20-25 wherein said agent is a polypeptide.

33. A method according to any of Claims 20-25 wherein said agent is a peptide.

34. A method according to any of Claims 20-25 wherein said agent is nucleic acid molecule.

35. A method according to Claim 34 wherein said nucleic acid molecule is an aptamer.

36. A method according to Claim 34 wherein said nucleic acid is an inhibitory RNA molecule.

37. A method according to Claim 36 wherein said inhibitory RNA is encoded by a transcription cassette comprising a nucleic acid molecule, or part thereof, selected from the group consisting of:

i) a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1;

ii) a nucleic acid molecule which hybridises to the sequence in (i); or

iii) a nucleic acid molecule which is degenerate because of the genetic code to the sequences defined in (i) and (ii) above; wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

38. A method according to Claim 37 wherein said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

39. A method according to Claim 37 wherein said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

40. A method according to Claim 34 wherein said nucleic acid molecule is an antisense nucleic acid molecule.

41. An antibody, or effective binding part thereof, identified by the method according to any of Claims 26-31 for use as a pharmaceutical.

42. A polypeptide identified by the method according to Claim 32 for use as a pharmaceutical.

43. A peptide identified by the method according to Claim 33 for use as a pharmaceutical.

44. A nucleic acid molecule identified by the method according Claim 34 for use as a pharmaceutical.

45. Use according to Claim 44 wherein said nucleic acid molecule is an aptamer.

46. Use according to Claim 44 wherein said nucleic acid molecule is an inhibitory RNA.

47. Use according to Claim 44 wherein said nucleic acid molecule is an antisense nucleic acid molecule.

48. Use according to any of Claims 41-47 wherein said pharmaceutical further comprises a a diluent, carrier or excipient.

Abstract

We describe a method for the identification of genes which show regulated expression in response to carbon source utilisation, typically genes associated with the initiation and/or promotion of cell transformation from a non-cancerous to a cancerous phenotype, typically of cells found in the colon; the use of these genes in diagnostic assays and as targets for the development of chemotherapeutic drugs and agents identified by said assay.

TABLE 1

```
AC   J02966;
DE   Human mitochondrial ADP/ADT translocator mRNA, complete cds.
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KW   ADP/ADT translocator.
/translation="MGDHAWSFLKDFLAGAVAAAVSKTAVAPIERVKLLLQVQHASKQI
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FT                    RHKQFWRYFAGNLASGGAAGATSLCFVYPLDFARTRLAADVGRRAQREFHGLGDCIIKI
FT                    FKSDGLRGLYQGFNVSVQGIIIYRAAYFGVYDTAKGMLPDPKNVHIFVSWMIAQSVTAV
FT                    AGLLSYPFDTVRRRMMMQSGRKGADIMYTGTVDCWRKIAKDEGAKAFFKGAWSNVLRGM
FT                    GGAFVLVLYDEIKKYV"
XX
SQ   Sequence 1320 BP; 341 A; 304 C; 357 G; 318 T; 0 other;
     cccccctagcg tcgcgcaggg tcggggactg cgcgcggtgc caggccgggc gtgggcgaga        60
     gcacgaacgg gctgctgcgg gctgagagcg tcgagctgtc accatgggtg atcacgcttg       120
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     ccacagcaaa tagaaataat ttatcatact tgtacaatta actgaagaat tgataataac      1260
     tgaatgtgaa acatcaataa agaccactta atgcacaaaa aaaaaaaaaa aaaaaaaaaa      1320
//
```

HSA132099 standard; mRNA; HUM; 3109 BP.
Homo sapiens mRNA for VNN1 protein

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Sequence 3109 BP; 973 A; 630 C; 601 G; 905 T; 0 other;
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aagcacctca gaatgtgact gtatttggag acagggtctt taaagaggta aaataaggtc 2100
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gcactgtgca aagcatgccc ttctgcacag gagagcaagg cacttgcagt agtgatctat 2820
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ggaatcctca gagcaattct ctgtattctc atataatttc aatgtaaaac agaaaacata 3060
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Homo sapiens transmembrane protein 5, mRNA (cDNA clone MGC:17085 IMAGE:3919181), complete cds.

/protein_id="AAH13152.1"
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GAAPARERRGREQSTLESEEWNPWEGDEKNEQQHRFKTSLQILDKSTKGKTDLSVQIWG
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ELLFIIYDSPWINDVDVFQWPLGVATYRNFPVVEASWSMLHDERPYLCNFLGTIYENSS
RQALMNILKKDGNDKLCWVSAREHWQPQETNESLKNYQDALLQSDLTLCPVGVNTECYR
IYEACSYGSIPVVEDVMTAGNCGNTSVHHGAPLQLLKSMGAPFIFIKNWKELPAVLEKE
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Sequence 1469 BP; 446 A; 300 C; 349 G; 374 T; 0 other;
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agaaaaatgt tacttcagtg gtatcagcac ttcaagacag agcttaaaat gaaatttact 1380
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DE   Homo sapiens CD3e-associated protein (CAST) mRNA, complete cds.
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FT                   /translation="MEEPQAGGEDAARFSCPPNFTAKPPASESPRFSLEALTGPDTELW
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FT                   GGLTCASAPQGTLRILEGPQQSLSGSPLQPIPASPPPQIPPGLRPRFCAFGGNPPVTGP
FT                   RSALAPNLLTSGKKKKEMQVTEAPVTQEAVNGHGALEVDMALGSPEMDVRKKKKKKNQQ
FT                   LKEPEAAGPVGTEPTVETLEPLGVLFPSTTKKRKKPKGKETFEPEDKTVKQEQINTEPL
FT                   EDTVLSPTKKRKRQKGTEGMEPEEGVTVESQPQVKVEPLEEAIPLPPTKKRKKEKGQMA
FT                   MMEPGTEAMEPVEPEMKPLESPGGTMAPQQPEGAKPQAQAALAAPKKKTKKEKQQDATV
FT                   EPETEVVGPELPDDLEPQAAPTSTKKKKKKKERGHTVTEPIQPLEPELPGEGQPEARAT
FT                   PGSTKKRKKQSQESRMPETVPQEEMPGPPLNSESGEEAPTGRDKKRKQQQQQPV"
XX
SQ   Sequence 1841 BP; 512 A; 502 C; 576 G; 251 T; 0 other;
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     aactttaccg cgaagccccc agcctcagag tcccctcgtt tctccttgga ggcgctgacg       120
     ggtccagata cggagctgtg gcttattcag gcccctgcag actttgcccc agaatgcttc       180
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     ccagtcacag ggcctaggtc agccttggcc cccaacctgc tcacctcagg gaagaagaaa       540
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     ctggaggtgg acatggcttt ggggtcgcca gaaatggatg tgcggaagaa gaagaagaaa       660
     aaaaatcagc agctgaaaga accagaggca gcagggcctg tggggacaga gcccacagtg       720
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     gagcctctag aagacacagt cctgtccccg accaaaaaga gaaagaggca aaggggacg        900
     gaagggatgg agccagagga gggggtgaca gttgagtctc agccacaggt gaaggtggag       960
     ccactggagg aagccatccc tctgcccccct acgaagaaga ggaaaaaaga aaagggacag     1020
     atggcaatga tggagccagg gacggaggcg atggagccag tggagccgga gatgaagcct      1080
     ctggagtccc caggggggac catggcgcct caacagccag aaggagcgaa gcctcaggcc      1140
     caggcagctc tggcagctcc caaaaagaag acgaagaaag aaaaacagca agatgccaca      1200
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     gctcccacat ccaccaagaa gaagaagaag aagaaagaga gaggtcacac agtgactgag      1320
     ccaattcagc cactagagcc tgaactgcca ggggagggac agcctgaagc cagggcaact      1380
     ccgggatcca ccaagaagag gaagaagcag agtcaggaaa gccggatgcc agagacagtg      1440
     ccccaagagg agatgccagg gccgccactg aattcagagt ctggggagga ggctcccaca      1500
     ggccgggaca agaagcggaa gcagcagcag cagcagcctg tgtagtctgc cccccgggaaa     1560
     ctgaggaact aaagaaagct gaaggtgccc acctgggcca ccagaaggtg acacccccag      1620
     aatccctccc cagagactgc accagcgcag ccagcaggag cctggcctgg gaggacgatt      1680
     tattattaca ctgggggttt ccttggcagc tggggtcatc agggtacttt caagaagggc      1740
     tcgtgcagga catcaaacag cctccgggcc tggatgggag ggagaaaaaa atgaggaacc      1800
agtcattaaa ggagctgttt cctgggtaaa aaaaaaaaa a
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Homo sapiens Apo-2 ligand mRNA, complete cds.

/translation="MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQM
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EKQQNISPLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSF
LSNLHLRNGELVIHEKGFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILL
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3'UTR 937..1042

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Sequence 1042 BP; 348 A; 208 C; 232 G; 254 T; 0 other;
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agcctgggac agacctgcgt gctgatcgtg atcttcacag tgctcctgca gtctctctgt    180
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aaaagtggca ttgcttgttt cttaaaagaa gatgacagtt attgggaccc caatgacgaa    300
gagagtatga acagcccctg ctggcaagtc aagtggcaac tccgtcagct cgttagaaag    360
atgattttga gaacctctga ggaaaccatt tctacagttc aagaaaagca acaaaatatt    420
tctcccctag tgagagaaag aggtcctcag agagtagcag ctcacataac tgggaccaga    480
ggaagaagca acacattgtc ttctccaaac tccaagaatg aaaaggctct gggccgcaaa    540
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cgatttcagg aggaaataaa agaaaacaca aagaacgaca aacaaatggt ccaatatatt    720
tacaaataca caagttatcc tgaccctata ttgttgatga aaagtgctag aaatagttgt    780
tggtctaaag atgcagaata tggactctat tccatctatc aagggggaat atttgagctt    840
aaggaaaatg acagaatttt tgtttctgta acaaatgagc acttgataga catggaccat    900
gaagccagtt ttttcggggc ctttttagtt ggctaactga cctggaaaga aaaagcaata    960
acctcaaagt gactattcag ttttcaggat gatacactat gaagatgttt caaaaaatct   1020
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DE   Homo sapiens mRNA for annexin A13 (ANXA13 gene), isoform b

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FT                   /translation="MGNRHSQSYTLSEGSQQLPKGDSQPSTVVQPLSHPSRNGEPEAPQ
FT                   PAKASSPQGFDVDRDAKKLNKACKGMGTNEAAIIEILSGRTSDERQQIKQKYKATYGKE
FT                   LEEVLKSELSGNFEKTALALLDRPSEYAARQLQKAMKGLGTDESVLIEVLCTRTNKEII
FT                   AIKEAYQRLFDRSLESDVKGDTSGNLKKILVSLLQANRNEGDDVDKDLAGQDAKDLYDA
FT                   GEGRWGTDELAFNEVLAKRSYKQLRATFQAYQILIGKDIEEAIEEETSGDLQKAYLTLV
FT                   RCAQDCEDYFAERLYKSMKGAGTDEETLIRIVVTRAEVDLQGIKAKFQEKYQKSLSDMV
FT                   RSDTSGDFRKLLVALLH"
FT   exon            84..206
FT                   /gene="ANXA13"
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     tgcctaaagg ggactcccaa ccctcgacag tcgtgcagcc tctcagccac ccatcacgga       180
     atggagagcc agaggcccca cagcctgcta aagcgagcag tcctcagggt tttgatgtgg       240
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     aatcagatgt caaaggtgat acaagtggaa acctaaaaaa aatcctggtg tctctgctgc       660
     aggctaatcg caatgaagga gatgacgtgg acaaagatct agctggtcag gatgccaaag       720
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     tggccaagag gagctacaag cagttacgag ccacctttca agcctatcaa attctcattg       840
     gcaaagacat agaagaagcc attgaagaag aaacatcagg cgacttgcag aaggcctatt       900
     taactctcgt gagatgtgcc caggattgtg aggactattt tgctgaacgt ctgtacaagt       960
     cgatgaaggg tgcggggacc gatgaggaga cgttgattcg catagtcgtg accagggccg      1020
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     actatgcatt tgtaatggca catttagaag gatatgcatc acacaagtaa ggtacaggaa      1500
     agacaaaatt aaacaattta ttaattttcc ttctgtgtgt tcaatttgaa agcctcattg      1560
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)E Homo sapiens serine protease inhibitor, Kazal type 1, mRNA (cDNA clone

Sequence 362 BP; 121 A; 74 C; 75 G; 92 T; 0 other;

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cttaatggat gcaccaagat atatgaccct gtctgtggga ctgatggaaa tacttatccc      180
aatgaatgcg tgttatgttt tgaaaatcgg aaacgccaga cttctatcct cattcaaaaa      240
tctgggcctt gctgagaacc aaggttttga aatcccatca ggtcaccgcg aggcctgact      300
ggccttattg ttgaataaat gtatctgaat atcaaaaaaa aaaaaaaaaa aaaaaaaaaa      360
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DE   Homo sapiens B cell linker protein BLNK mRNA, alternatively spliced,
DE   complete cds.

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FT                   TRPVHPALPFARGEYIDNRSSQRHSPPFSKTLPSKPSWPSEKARLTSTLPALTALQKPQ
FT                   VPPKPKGLLEDEADYVVPVEDNDENYIHPTESSSPPPEKAPMVNRSTKPNSSTPASPPG
FT                   TASGRNSGAWETKSPPPAAPSPLPRAGKKPTTPLKTTPVASQQNASSVCEEKPIPAERH
FT                   RGSSHRQEAVQSPVFPPAQKQIHQKPIPLPRFTEGGNPTVDGPLPSFSSNSTISEQEAG
FT                   VLCKPWYAGACDRKSAEEALHRSNKDGSFLIRKSSGHDSKQPYTLVVFFNKRVYNIPVR
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XX
SQ   Sequence 1806 BP; 571 A; 448 C; 379 G; 408 T; 0 other;
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     ccacccttca gcaagacact tcccagtaag cccagctggc cttcagagaa agcaaggctc       600
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     accaagccaa attcctcaac gcccgcctct cctccaggaa cagcttcagg tcgaaacagt       840
     ggggcctggg aaaccaagtc acctccacca gctgcaccat ccccgttgcc acgggccggg       900
     aaaaaaccaa cgacaccact gaagacaact ccagttgcct ctcaacagaa tgcttcaagt       960
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     ctgccaagat ttacagaagg gggaaaccca actgtggatg ggcccctacc cagcttttca      1140
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     aataagcgag tatataatat tcctgtgcga tttattgaag caacaaaaca atatgccttg      1380
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     caacatagtc ctttggttct tattgacagt cagaataaca caaaagattc caccagactg      1500
     aagtatgcag ttaaagtttc ataaaggggg aaaaaaaaga tcaataccat tgcttcagac      1560
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Homo sapiens cDNA FLJ12768 fis, clone NT2RP2001576, weakly similar to HYPOTHETICAL 62.2 KD PROTEIN C4G8.12C IN CHROMOSOME I.

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GFFNRPTFLAFAVVPLYLWGTRGATNPGLKSLTREALVLLPGATLTAAVFVATDSWYFS
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Sequence 2687 BP; 454 A; 883 C; 733 G; 617 T; 0 other;
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Homo sapiens glycine amidinotransferase (L-arginine:glycine amidinotransferase), mRNA (cDNA clone MGC:1744 IMAGE:3010128), complete

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Sequence 2342 BP; 690 A; 490 C; 480 G; 682 T; 0 other;
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aa

)E Homo sapiens cDNA FLJ10143 fis, clone HEMBA1003281, weakly similar to
)E POLIOVIRUS RECEPTOR PRECURSOR.

'T /translation="MGTQEGWCLLLCLALSGAAETKPHPAEGQWRAVDVVLDCFLAKDG
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'T GGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVTHISLEEPLGASTQV
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Q Sequence 1694 BP; 365 A; 514 C; 488 G; 327 T; 0 other;

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DE Homo sapiens leucine aminopeptidase 3, mRNA (cDNA clone IMAGE:2821948), partial cds.

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Sequence 1938 BP; 603 A; 386 C; 470 G; 479 T; 0 other;
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DE   gene)

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Human mRNA for (2'-5') oligo A synthetase E (1,6 kb RNA)

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Sequence 1322 BP; 334 A; 353 C; 320 G; 315 T; 0 other;

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04DEC03 E856959-2 D02973
P01/7700 0.00-0328048.4

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The Patent Office

Cardiff Road
Newport
South Wales
NP9 1RH

1.	Your reference	P104199GB
2.	Patent application number *(The Patent Office will fill in this part)*	0328048.4
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	University of Sheffield Western Bank Sheffield S10 2TN GB
	Patents ADP number *(if you know it)*	7397683 1001
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	Gene Screen
5.	Name of your agent *(if you have one)*	Harrison Goddard Foote
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	31 St Saviourgate YORK YO1 8NQ
	Patents ADP number *(if you know it)*	07914237002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* — yes

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

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Continuation sheets of this form	
Description	299 (tables 1+2 added to description)
Claim *(s)*	7 CF
Abstract	1
Drawing *(s)*	2+2

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	tables 1+2 added to description.

11\. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 3/12/03

12. Name and daytime telephone number of person to contact in the United Kingdom   Rob Docherty   01904 732120

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DUPLICATE

Gene Screen

The invention relates to a screen for the identification of genes which show regulated expression in response to carbon source utilisation.

Colorectal cancer is a cancer which occurs in the large intestine and rectum. The colon can be divided into effectively four sections; the ascending colon; the transverse colon; the descending colon; and the sigmoid colon. Most colorectal cancers arise in the sigmoid colon and develop from "polyps" which can grow for several years before becoming cancerous. The early detection of these pre-cancerous growths is obviously desirable since removal of the polyps is a very effective means to stem the progress of disease.

There are various types of colorectal cancer. Most cancers of this type are adenocarcinomas which are malignant growths which begin in the epithelial cells which line the colon and rectum. Other cancers of the colon and rectum include gastrointestinal stromal tumours and lymphomas. In some examples the patient can be asymptomatic and for this reason it is important that screening is undertaken to identify those patients in which pre-cancerous polyps are forming. However, some patients do present with symptoms and these include rectal bleeding, diarrhoea, constipation, abdominal pain, and general weakness.

As mentioned above, regular screening is by far the most effective way of controlling this disease since removal of pre-cancerous polyps by surgery can effectively cure any disease before it is initiated. Currently, diagnostic tests include the use of colonoscopy, which allows a doctor to examine the rectum and colon; faecal blood analysis to check for any bleeding from the bowel and rectal area although this test is not directly diagnostic for cancerous lesion in its own right; and sigmoidoscopy which is similar to colonoscopy but only investigates the lower bowel area. Typically, patients with a family history of colorectal cancer can be expected to have

a colonoscopy every 5 years or so and a blood stool check on a yearly basis from about the age of 40.

The treatment of colorectal cancer usually involves invasive surgery to remove polyps and/or malignant growths. If the cancer has developed beyond the polyp stage then more extensive surgery is required which can result in removal of part of the bowel and surrounding lymph nodes. In the situation where a cancer necessitates extensive surgery a colostomy stoma may be required, at least for a period, to allow the bowel to recover from surgery. Surgery in the rectal region is more complicated and is largely dependent on how far the disease has progressed. In some cases the surgery can damage nerves which control sexual and urinary functions. In advanced stage colorectal cancers metastatic lesions may require removal and in about 15% of cases the lesions are in the liver which requires removal of large parts of the liver. The surgical removal of polyps and/or cancerous growths lead to a good prognosis for patients. In some cases surgery is followed by a course of chemotherapy (for colon cancer) and chemotherapy and radiation therapy (rectal cancer) to remove any cancer cells not detected during surgery. The chemotherapeutic agents typically used to treat colorectal cancer include 5-fluorouracil, leucovorin, irinotecan and capecitabine.

It is apparent that the early detection of cells which are pre-cancerous is highly desirable since in most cases surgery to remove these cells results in a very good prognosis for patients. Diagnostic tests which use the detection of cancer markers as an early indicator of cancer are known in the art.

For example, EP1355149 describes gene expression profiles from colorectal samples to provide a “finger print” expression profile as an indication of whether a patient is susceptible to the development of colorectal cancer or indeed if malignant growth has already been initiated. The disclosure in EP1355149 is directed to the use of microarrays to compare transformed and non-transformed tissue gene expression in a global sense.

WO02/059609 also describes a gene screen which utilises expression profiles in breast and colorectal cancer. A comparison is made between "normal" and "abnormal" samples in patients to provide a global picture of gene expression in these samples as an indicator of particular genes which are either over-expressed or abrogated between samples. Both EP1355149 and WO02/059609 take a shot gun approach to screening for target genes which can be used either as a diagnostic tool or as a target for the development of new chemotherapeutic agents.

The present invention provides a targeted screen for genes the expression of which may be altered in a response to carbon source. The invention makes use of the differences in expression profiles between normal and diseased tissue as a consequence of differences in metabolic state between cancer cells and normal cells due in part to carbon source utilisation by these respective cell types. The epithelial cells which line the colon and rectum metabolise butyrate as a carbon source for energy transduction via glycolysis. The main carbon source utilised by tumour cells is glucose. Consequently, expression profiles between these cell types are different due to the differences in carbon source metabolism.

We have identified a large number of potential markers of colorectal cancer which have utility with respect to the early diagnosis of disease and as targets for the development of novel chemotherapeutic agents. Moreover, this assay has broader applicability to conditions resulting from dysfunction of the bowel (e.g colitis, ulcerative colitis, diversion colitis. Crohn's disease and irritable bowel syndrome. In addition the assay provides a screening tool for fibre consumption and as an assay for colon microflora functionality (the effectiveness of fermentation of specific fibres).

According to an aspect of the invention there is provided a method to screen for nucleic acid molecules which show altered expression in an isolated first cell sample comprising comparing the gene expression profiles between said first cell sample with a second reference cell sample wherein said first cell sample has been grown in

the presence of the carbon source butyrate, or a related carbon source from which butyrate is derived, either directly or indirectly, and comparing said expression profile with the expression profile in said second reference cell sample which has not been grown in the presence of butyrate, or said related carbon source.

According to a further aspect of the invention there is provided a method to screen for nucleic acid molecules which show altered expression in an isolated biological sample comprising the steps of:

i) providing
   a) a cell growth preparation comprising a first cell sample derived from at least one region of the colon; cell growth media; and a carbon source wherein said carbon source is butyrate; and
   b) a cell growth preparation comprising a second cell sample derived from an equivalent region of the colon; cell growth media; and a carbon source which is not butyrate;

ii) extracting nucleic acid from said first and second cell samples; and

iii) comparing the gene expression profile in said first cell sample with the gene expression profile in said second cell sample.

In a preferred method of the invention said first and second cell samples are derived from the ascending colon.

In an alternative preferred method of the invention said first and second cell samples are derived from the transverse colon.

In a further preferred method of the invention said first and second samples are derived from the descending colon.

In a still further preferred method of the invention said first and second samples are derived from the sigmoid region of the colon. Preferably said cell samples are derived from the rectal region of the colon.

In a further preferred method of the invention said first and second cell samples comprise epithelial cells.

In a preferred method of the invention said carbon source which is not butyrate is glucose.

In a still further preferred method of the invention said nucleic acid molecule which shows altered expression is selected from the group as represented by the nucleic acid sequences shown in Table 1, or nucleic acid molecules which hybridise to the sequences presented Table 1. Preferably said nucleic acid molecules hybridise under stringent hybridisation conditions.

According to a further aspect of the invention there is provided a method for the detection of at least one nucleic acid molecule associated with the initiation and/or progression of colorectal cancer, in an animal, comprising the steps of:

i) providing a biological sample comprising at least one cell to be tested;

ii) contacting said sample with a ligand which binds at least one nucleic acid molecule as represented by the nucleic acid sequence selected from the group consisting of:

  a) a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1;

  b) a nucleic acid molecule which hybridises to nucleic acid molecules as defined in (a);

  c) a nucleic acid molecule that is degenerate as a consequence of the genetic code to the nucleic acid molecule represented in (a) and (b);

iii) detecting the presence of at least one nucleic acid molecule in said sample.

In a preferred method of the invention said animal is human.

In a further preferred method of the invention said colorectal cancer is adenocarcinoma.

In a preferred method of the invention said ligand is a nucleic acid molecule adapted to anneal to said nucleic acid molecule which is indicative of colorectal cancer.

It will be apparent to the skilled person that a number of nucleic acid based assay systems are available which can be adapted to detect nucleic acid molecules as hereindisclosed. For example quantitative polymerase chain reaction assays, *in situ* hybridisation, northern blot.

According to a further aspect of the invention there is provided a method for the detection of at least one polypeptide associated with the initiation and/or progression of colorectal cancer, in an animal, comprising the steps of:

i) providing a biological sample comprising at least one cell to be tested;

ii) contacting said sample with at least one ligand which ligand specifically binds at least one polypeptide encoded by a nucleic acid molecule as represented by the nucleic acid sequence shown in Table 1, or a variant polypeptide comprising an amino acid sequence which varies by the addition, deletion or substitution of at least one amino acid residue; and

iii) detecting the presence of at least one polypeptide in said sample.

In a preferred method of the invention said animal is human.

In a further preferred embodiment of the invention said ligand is an antibody, preferably a monoclonal antibody, or at least the effective binding part thereof.

Methods which utilise antibodies to detect the presence of a polypeptide in a biological sample are well known in the art and include ELISA's, western blot and immunofluoresence.

According to a further aspect of the invention there is provided the use of at least one polypeptide, or variant sequence thereof, encoded by a nucleic acid molecule(s) as represented by the nucleic acid sequences as shown in Table 1, as a target for the screening of agents which modulate the activity of said polypeptide.

According to a yet further aspect of the invention there is provided a method to screen for agents which modulate the activity of at least one gene associated with the initiation and/or progression of colorectal cancer comprising the steps of:

i) forming a preparation comprising at least one polypeptide wherein said polypeptide is encoded by a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1, or a variant polypeptide comprising an amino acid sequence which varies by the addition, deletion or substitution of at least one amino acid residue as represented by the amino acid sequences shown in Table 1, and at least one agent to be tested; and

ii) determining the activity of said agent with respect to activity of said polypeptide.

In a preferred method of the invention said polypeptide is expressed by a cell wherein said cell is transformed or transfected with said nucleic acid molecule. Preferably said nucleic acid molecule is part of a vector adapted for recombinant expression of said nucleic acid molecule. Preferably said vector is provided with a promoter which enables the expression of said nucleic acid molecule to be regulated.

In a preferred method of the invention said cell is derived from the colon, preferably said cell is an epithelial cell which lines said colon.

In a further preferred method of the invention said agent is an antibody, preferably a monoclonal antibody or modified antibody, or at least the effective binding part thereof.

Antibodies, also known as immunoglobulins, are protein molecules which usually have specificity for foreign molecules (antigens). Immunoglobulins (Ig) are a class of structurally related proteins consisting of two pairs of polypeptide chains, one pair of light (L) (low molecular weight) chain (κ or λ), and one pair of heavy (H) chains (γ, α, μ, δ and ε), all four linked together by disulphide bonds. Both H and L chains have regions that contribute to the binding of antigen and that are highly variable from one Ig molecule to another. In addition, H and L chains contain regions that are non-variable or constant.

The L chains consist of two domains. The carboxy-terminal domain is essentially identical among L chains of a given type and is referred to as the "constant" (C) region. The amino terminal domain varies from L chain to L chain and contributes to the binding site of the antibody. Because of its variability, it is referred to as the "variable" (V) region.

The H chains of Ig molecules are of several classes, α, μ, σ, α, and γ (of which there are several sub-classes). An assembled Ig molecule consisting of one or more units of two identical H and L chains, derives its name from the H chain that it possesses. Thus, there are five Ig isotypes: IgA, IgM, IgD, IgE and IgG (with four sub-classes based on the differences in the 'constant' regions of the H chains, i.e., IgG1, IgG2, IgG3 and IgG4). Further detail regarding antibody structure and their various functions can be found in, Using Antibodies: A laboratory manual, Cold Spring Harbour Laboratory Press.

In a preferred method of the invention said fragment is a Fab fragment.

In a further preferred method of the invention said antibody is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; and antibodies comprising CDR3 regions.

Preferably said fragments are single chain antibody variable regions (scFV's) or domain antibodies. If a hybridoma exists for a specific monoclonal antibody it is well within the knowledge of the skilled person to isolate scFv's from mRNA extracted from said hybridoma via RT PCR. Alternatively, phage display screening can be undertaken to identify clones expressing scFv's. Domain antibodies are the smallest binding part of an antibody (approximately 13kDa). Examples of this technology is disclosed in US6, 248, 516, US6, 291, 158, US6,127, 197 and EP0368684 which are all incorporated by reference in their entirety.

A modified antibody, or variant antibody and reference antibody, may differ in amino acid sequence by one or more substitutions, additions, deletions, truncations which may be present in any combination. Among preferred variants are those that vary from a reference polypeptide by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid by another amino acid of like characteristics. The following non-limiting list of amino acids are considered conservative replacements (similar): a) alanine, serine, and threonine; b) glutamic acid and asparatic acid; c) asparagine and glutamine d) arginine and lysine; e) isoleucine, leucine, methionine and valine and f) phenylalanine, tyrosine and tryptophan. Most highly preferred are variants which show enhanced biological activity.

Preferably said antibody is a humanised or chimeric antibody.

A chimeric antibody is produced by recombinant methods to contain the variable region of an antibody with an invariant or constant region of a human antibody.

A humanised antibody is produced by recombinant methods to combine the complementarity determining regions (CDRs) of an antibody with both the constant (C) regions and the framework regions from the variable (V) regions of a human antibody.

Chimeric antibodies are recombinant antibodies in which all of the V-regions of a mouse or rat antibody are combined with human antibody C-regions. Humanised antibodies are recombinant hybrid antibodies which fuse the complimentarity determining regions from a rodent antibody V-region with the framework regions from the human antibody V-regions. The C-regions from the human antibody are also used. The complimentarity determining regions (CDRs) are the regions within the N-terminal domain of both the heavy and light chain of the antibody to where the majority of the variation of the V-region is restricted. These regions form loops at the surface of the antibody molecule. These loops provide the binding surface between the antibody and antigen.

Antibodies from non-human animals provoke an immune response to the foreign antibody and its removal from the circulation. Both chimeric and humanised antibodies have reduced antigenicity when injected to a human subject because there is a reduced amount of rodent (i.e. foreign) antibody within the recombinant hybrid antibody, while the human antibody regions do not elicit an immune response. This results in a weaker immune response and a decrease in the clearance of the antibody. This is clearly desirable when using therapeutic antibodies in the treatment of human diseases. Humanised antibodies are designed to have less "foreign" antibody regions and are therefore thought to be less immunogenic than chimeric antibodies.

In an alternative preferred method of the invention said agent is a polypeptide or a peptide. Preferably said polypeptide or peptide is modified.

In a preferred method of the invention said peptide is at least 6 amino acid residues in length. Preferaby the length of said peptide/polypeptide is selected from the group

consisting of: at least 7 amino acid residues; 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues in length. Alternatively the length of said peptide/polypeptide is at least 20 amino acid residues; 30; 40; 50; 60; 70; 80; 90; or 100 amino acid residues in length.

It will be apparent to one skilled in the art that modification to the amino acid sequence of peptide agents could enhance the binding and/or stability of the peptide with respect to its target sequence. In addition, modification of the peptide may also increase the *in vivo* stability of the peptide thereby reducing the effective amount of peptide necessary to inhibit the activity of a target polypeptide. This would advantageously reduce undesirable side effects which may result *in vivo*. Alternatively or preferably, said modification includes the use of modified amino acids in the production of recombinant or synthetic forms of peptides. It will be apparent to one skilled in the art that modified amino acids include, by way of example and not by way of limitation, 4-hydroxyproline, 5-hydroxylysine, N^6-acetyllysine, N^6-methyllysine, N^6,N^6-dimethyllysine, N^6,N^6,N^6-trimethyllysine, cyclohexyalanine, D-amino acids, ornithine. Other modifications include amino acids with a C_2, C_3 or C_4 alkyl R group optionally substituted by 1, 2 or 3 substituents selected from halo (e.g. F, Br, I), hydroxy or C_1-C_4 alkoxy. Modifications also include, by example and not by way of limitation, acetylation and amidation.

In a preferred embodiment of the invention said peptide sequence is acetylated. Preferably said acetylation is to the amino terminus of said peptide.

In a further preferred embodiment of the invention said peptide sequence is amidated. Preferably said amidation is to the carboxyl-terminus of said peptide.

It will also be apparent to one skilled in the art that peptides could be modified by cyclisation. Cyclisation is known in the art, (see Scott *et al* Chem Biol (2001), 8:801-815; Gellerman et al J. Peptide Res (2001), 57: 277-291; Dutta *et al* J. Peptide

Res (2000), 8: 398-412; Ngoka and Gross J Amer Soc Mass Spec (1999), 10:360-363.

In a further preferred method of the invention said agent is nucleic acid molecule. Preferably said nucleic acid molecule is an aptamer or a modified aptamer. In an alternative preferred method of the invention said nucleic acid is an inhibitory RNA (RNAi) molecule. Alternatively said nucleic acid molecule is an antisense nucleic acid molecule.

Nucleic acids have both linear sequence structure and a three dimensional structure which in part is determined by the linear sequence and also the environment in which these molecules are located. Conventional therapeutic molecules are small molecules, for example, peptides, polypeptides, or antibodies, which bind target molecules to produce an agonistic or antagonistic effect. It has become apparent that nucleic acid molecules also have potential with respect to providing agents with the requisite binding properties which may have therapeutic utility. These nucleic acid molecules are typically referred to as aptamers. Aptamers are small, usually stablised, nucleic acid molecules which comprise a binding domain for a target molecule. A screening method to identify aptamers is described in US 5,270,163, which is incorporated by reference. Aptamers are typically oligonucleotides which may be single stranded oligodeoxynucleotides, oligoribonucleotides, or modified oligodeoxynucleotide or oligoribonucleotides.

The term "modified" encompasses nucleotides with a covalently modified base and/or sugar. For example, modified nucleotides include nucleotides having sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified nucleotides may also include 2' substituted sugars such as 2'-O-methyl-; 2-O-alkyl; 2-O-allyl; 2'-S-alkyl; 2'-S-allyl; 2'- fluoro-; 2'-halo or 2;azido-ribose, carbocyclic sugar analogues a-anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, and sedoheptulose.

Modified nucleotides are known in the art and include by example and not by way of limitation; alkylated purines and/or pyrimidines; acylated purines and/or pyrimidines; or other heterocycles. These classes of pyrimidines and purines are known in the art and include, pseudoisocytosine; N4, N4-ethanocytosine; 8-hydroxy-N6-methyladenine; 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil; 5-fluorouracil; 5-bromouracil; 5-carboxymethylaminomethyl-2-thiouracil; 5-carboxymethylaminomethyl uracil; dihydrouracil; inosine; N6-isopentyl-adenine; 1-methyladenine; 1-methylpseudouracil; 1-methylguanine; 2,2-dimethylguanine; 2-methyladenine; 2-methylguanine; 3-methylcytosine; 5-methylcytosine; N6-methyladenine; 7-methylguanine; 5- methylaminomethyl uracil; 5-methoxy amino methyl-2-thiouracil; β-D-mannosylqueosine; 5-methoxycarbonylmethyluracil; 5-methoxyuracil; 2 methylthio-N6-isopentenyladenine; uracil-5-oxyacetic acid methyl ester; psueouracil; 2-thiocytosine; 5-methyl-2 thiouracil, 2-thiouracil; 4-thiouracil; 5-methyluracil; N-uracil-5-oxyacetic acid methylester; uracil 5—oxyacetic acid; queosine; 2-thiocytosine; 5-propyluracil; 5-propylcytosine; 5-ethyluracil; 5-ethylcytosine; 5-butyluracil; 5-pentyluracil; 5-pentylcytosine; and 2,6,-diaminopurine; methylpsuedouracil; 1-methylguanine; 1-methylcytosine.

The aptamers of the invention are synthesized using conventional phosphodiester linked nucleotides and synthesized using standard solid or solution phase synthesis techniques which are known in the art. Linkages between nucleotides may use alternative linking molecules. For example, linking groups of the formula P(O)S, (thioate); P(S)S, (dithioate); P(O)NR'2; P(O)R'; P(O)OR6; CO; or CONR'2 wherein R is H (or a salt) or alkyl (1-12C) and R6 is alkyl (1-9C) is joined to adjacent nucleotides through –O- or –S-. The binding of aptamers to a target polypeptide is readily testable.

An alternative nucleic acid molecule is a so called RNAi molecule. A recent technique to specifically ablate gene function is through the introduction of double stranded RNA, also referred to as inhibitory RNA (RNAi), into a cell which results

in the destruction of mRNA complementary to the sequence included in the RNAi molecule. The RNAi molecule comprises two complementary strands of RNA (a sense strand and an antisense strand) annealed to each other to form a double stranded RNA molecule. The RNAi molecule is typically derived from exonic or coding sequence of the gene which is to be ablated. Recent studies suggest that RNAi molecules ranging from 100-1000bp derived from coding sequence are effective inhibitors of gene expression. Surprisingly, only a few molecules of RNAi are required to block gene expression which implies the mechanism is catalytic. The site of action appears to be nuclear as little if any RNAi is detectable in the cytoplasm of cells indicating that RNAi exerts its effect during mRNA synthesis or processing.

In a preferred method of the invention there is provided a cassette comprising a nucleic acid molecule, or part thereof, wherein said molecule is selected from the group consisting of:

i) a nucleic acid molecule represented by the nucleic acid sequence shown in Table 1 ;
ii) a nucleic acid molecule which hybridises to the sequence in (i) above and which encodes a polypeptide which initiates or promotes transformation of colon cells; or
iii) a nucleic acid molecule which is degenerate because of the genetic code to the sequences defined in (i) and (ii) above, wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

In a preferred method of the invention said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

In a further preferred method of the invention said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their

sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

In a preferred embodiment of the invention said first and second parts are linked by at least one nucleotide base.

In a preferred embodiment of the invention said first and second parts are linked by 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 nucleotide bases.

In a further preferred embodiment of the invention the length of the RNAi molecule is between 100bp-1000bp. More preferably still the length of RNAi is selected from 100bp; 200bp; 300bp; 400bp; 500bp; 600bp; 700bp; 800bp; 900bp; or 1000bp. More preferably still said RNAi is at least 1000bp.

In an alternative preferred method of the invention the RNAi molecule is between 15bp and 25bp, preferably said molecule is 21bp. Preferably said cassette is part of a vector.

According to a further aspect of the invention there is provided an antibody identified by the method according to the invention for use as a pharmaceutical.

According to a further aspect of the invention there is provided a polypeptide or peptide identified by the method according to the invention for use as a pharmaceutical.

According to a further aspect of the invention there is provided a nucleic acid molecule identified by the method according to the invention for use as a pharmaceutical.

In a preferred embodiment of the invention said nucleic acid molecule is an aptamer.

In an alternative preferred embodiment of the invention said nucleic acid molecule is an inhibitory RNA.

In a further alternative preferred embodiment of the invention said nucleic acid molecule is an antisense nucleic acid molecule.

In a preferred embodiment of the invention said pharmaceutical further comprises a a diluent, carrier or excipient.

When administered, the therapeutic compositions of the present invention are administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents, such as chemotherapeutic agents.

The therapeutics of the invention can be administered by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal. When antibodies are used therapeutically, a preferred route of administration is by pulmonary aerosol. Techniques for preparing aerosol delivery systems containing antibodies are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the antibodies, such as the paratope binding capacity (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing antibody aerosols without resort to undue experimentation. When using antisense preparations of the invention, slow intravenous administration is preferred.

The compositions of the invention are administered in effective amounts. An "effective amount" is that amount of a composition that alone, or together with further doses, produces the desired response. In the case of treating a particular disease, such as cancer, the desired response is inhibiting the progression of the disease. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine methods or can be monitored according to diagnostic methods of the invention discussed herein.

Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

The pharmaceutical compositions used in the foregoing methods preferably are sterile and contain an effective amount for producing the desired response in a unit of weight or volume suitable for administration to a patient. The response can, for example, be determined by measuring the physiological effects of the composition, such as regression of a tumour, decrease of disease symptoms, modulation of apoptosis, etc.

The doses of pharmaceutical agent administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of

administration used and the state of the subject. Other factors include the desired period of treatment. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits.

In general, doses of pharmaceutical are formulated and administered in doses between 1 ng and about 500mg, and between 10 ng and 100mg, according to any standard procedure in the art. Where nucleic acids are employed, doses of between 1 ng and 0.1mg generally will be formulated and administered according to standard procedures. Other protocols for the administration of compositions will be known to one of ordinary skill in the art, in which the dose amount, schedule of injections, sites of injections, mode of administration (e.g., intra-tumoral) and the like vary from the foregoing. Administration of pharmaceutical compositions to mammals other than humans, e.g. for testing purposes or veterinary therapeutic purposes, is carried out under substantially the same conditions as described above. A subject, as used herein, is a mammal, preferably a human, and including a non-human primate, cow, horse, pig, sheep, goat, dog, cat or rodent.

When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic,

malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

Pharmaceutcial compositions may be combined, if desired, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt.

The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. . All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous or non-aqueous preparation of pharmaceutical agents, which is preferably isotonic with the blood of the recipient. This preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

An embodiment of the invention will now be described by example only and with reference to the following Figures and Tables;

Figure 1 illustrates a concentration-response of cells growing in butyrate as sole carbon source. This is the summary of four independent repeat experiments. Legend shows butyrate concentrations in mM;

Figure 2 illustrates the purity and quality of RNA preparation. The 28S and 18S sample bands are tight and clearly resolved for RNA prepared from butyrate- and glucose-grown cells. Little or no DNA or salt contamination appears in the samples;

Table1 illustrates nucleic acid and protein sequences identified by the screening method according to the invention; and

Table 2 illustrates a summary of expression data of nucleic acid sequences identified in Table 1.

Materials and Methods

We have compared the expression profiles of colon cells growing in either glucose or butyrate as a carbon source. HT 29 colon carcinoma cells were cultured in DMEM medium (Gibco) in the presence of 10% foetal calf serum, penicillin and streptomycin. Cells were either cultured in glucose alone as the sole carbon source, or in butyrate as the sole extraneous provided carbon source. Empirical analysis of HT29 cells grown in multiple butyrate concentrations revealed that 2mM butyrate was optimal for cell culture in the absence of glucose. Cells were cultured in either medium for multiple passages (typically 4). RNA was extracted from cells grown in each condition and used to probe an Affymetrix human 12k array. The expression profile of cells cultured in each condition was compared and genes altered in expression by more than 2 fold are listed in Table 2.

Materials used during this study

ITEM	ITEM - SPECIFICS	SUPPLIER
Glucose medium (1)	Dulbecco's Modified Eagle Medium 25 mM HEPES 1 x 0.1 micron filtered with sodium pyruvate, with 1000	GIBCO

	mg/l glucose with pyridoxine + FCS + p/s (500 ml)	
Butyrate medium (2) 0.2 mM NaB medium	Dulbecco's Modified Eagle Medium 1 x 0.1 micron filtered with L-glutamine without glucose, without sodium pyruvate + NaB (1M) 110 μl + FCS + p/s (555.1 ml)	GIBCO
Butyrate medium (3) 2 mM NaB medium	Dulbecco's Modified Eagle Medium 1 x 0.1 micron filtered with L-glutamine without glucose, without sodium pyruvate + NaB (1M) 1100 μl + FCS + p/s (556.1 ml)	GIBCO
Medium without glucose and without butyrate (4)	Dulbecco's Modified Eagle Medium 1 x 0.1 micron filtered with L-glutamine without glucose, without sodium pyruvate + FCS + p/s (550 ml)	GIBCO
NaB stock	Sodium Butyrate powder dissolved in sterile water 250 mg in 2.27 ml water	Sigma

	(1M) 0.2 μm filter sterilised	
Sterile syringes	5 ml	Becton Dickinson UK, Ltd
Sterilising filters	0.2 μm Acrodisc	Gelman Sciences, Ltd
Item	**Item specifics**	**Supplier**
FCS	Foetal Calf Serum 50 ml per 500 ml DMEM	Harlan Sera Lab
P/S	Penicillin – Streptomycin solution 100ml bottle (100 X) – 5 ml per 500 ml DMEM	Sigma
TE for splitting cells	Trypsin Enzyme – 100 ml bottle - 3 ml per T75 and 1 ml per 6 well plate well	Sigma
FCS tubes	50 ml Centrifuge tubes	Corning Inc
P/S + TE tubes	30 ml Universal containers	Bibby Sterilin Ltd
Tissue Culture Plates	6 well sterile with lid single packed	Greiner bio-one
Tissue Culture Flasks	T 75	Nunclon
Stripette ® 5ml, 10ml,	Serological Pipette,	Corning Inc / Costar

25 ml	individually wrapped	
Pipette	Powerpette plus	Jencons
Cell Counting Slide	Haemocytometer, improved Neubauer	Neubauer
Ethanol for tissue culture	70 % EtOH	Sigma
Virkon for cell culture	1 % Virkon	Day Impex, Ltd
Microscope for cell work	Light 6 – 10X	CK Olympus, Tokyo
Paper towels	Blue	Jamont (UK), Ltd
Latex-free examination gloves	Large	Shermond Surgical Supply, Ltd
Item	**Item specifics**	**Supplier**
RNA extraction reagent	TRIzol ® Reagent	Invitrogen – Life technologies
RNA extraction reagent	Chloroform	Sigma
RNA extraction reagent	Isopropyl alcohol	Sigma

RNA extraction reagent	75% EtOH in DEPC-treated water	Sigma
RNA extraction reagent	Rnase-free water	Sigma
RNA clean up kit	Rneasy Midi Kit (10 RNeasy midi spin columns)	Qiagen
β- Mercaptoethanol	14.3 M stock solution	Sigma
Ethanol for Qiagen	96-100% EtOH	Sigma
Agarose	1g in 100 ml TB-EDTA-Buffer	Helena Biosciences, UK
TB-EDTA- Buffer	Tris-Borate-EDTA buffer 100ml	Sigma
Eppendorf tubes	1.5 ml	Sarstedt Laboratory supplies, Ltd
Loading buffer	6 X	Promega

The Human Colon Carcinoma Cell Line - HT29

The HT29 cell line is established from a colon adenocarcinoma which was removed from a 44 year old Caucasian woman. The cell line is epithelial in origin and hypertriploid. It has been shown to be tumourigenic in nude mice and synthesizes Carcino embryonic antigen - CEA (Egan & Todd, 1972) and the Transforming

growth factors - TGF-α and TGF-β (Anzano *et al.* 1989) when maintained *in vitro.* The HT29 cell line constitutively over-produces mutant p53 protein as a consequence of a point mutation at codon 273, resulting in an Arginine to Histidine amino acid substitution (Hsu *et al.* 1994).

The Culture of HT29 Colorectal adenocarcinoma cells

Cells were cultured in T75 tissue culture flasks (Nunclon) in 5% CO_2 at 37°C. Cells were passaged when confluent by washing twice in PBS and incubating in pre-warmed trypsin : EDTA (1:1) at 37°C until cells detached. The cells were then re-suspended in the appropriate growth medium, either glucose DMEM or butyrate DMEM before being seeded into new T75 tissue culture flasks or 6-well plates.

Optimisation of HT29 cell growth in butyrate as sole extraneous carbon source

HT29 cells were seeded out into 19 wells (in 6 well plates) at a cell density of 0.5 x 10^6 cells per well (i.e. 500 000 cells per well) deduced with the aid of a Haemocytometer (Improved Neubauer). These cells were taken from T75 - 0.2 mM butyrate (NaB) DMEM flasks and allowed to adhere to the 6-well plates over 72 hrs also in 0.2 mM NaB DMEM with FCS and Penicillin / Streptomycin antibiotics. After the cells had adhered to the surface of the 6 well plates the 0.2 mM NaB DMEM was removed and each well was washed twice with PBS in order to remove all traces of the 0.2 mM DMEM, then different concentrations of NaB DMEM with FCS and with Penicillin / Streptomycin antibiotics were added to the appropriate wells in triplicate. Cell counts were taken at various time points. Specific media was changed daily in order to maintain the appropriate / desired NaB concentrations per well. All solutions / reagents used were pre-warmed in a water bath prior to use so as to avoid any cold shock to the cells.

RNA extraction using TRIzol® Reagent

Total RNA was extracted from HT29 cells grown to confluence in T75 flasks using TRIzol Reagent as per manufacturer's recommendations. Cells were grown for several passages either in butyrate-containing medium, or in glucose-containing medium prior to extraction of RNA

Cells were homogenised using 1 ml TRIzol Reagent per 10 cm^2 area of culture surface. The homogenised samples were incubated for 5 minutes at at ambient temperature to permit the complete dissociation of nucleoprotein complexes. 200µl of chloroform was added to each sample. Tubes were shaken vigorously by hand for 15 seconds and incubated at ambient temperature for 3 minutes. Samples were centrifuged at 12000g for 15 minutes at 4oC. RNA in the aqueous phase was separated and precipitated using isopropyl alcohol. RNA was rinsed, air dried and redissolved in RNase-free water.

RNA was further purified using Qiagen RNeasy columns. The columns were used exactly as per manufacturers recommendations. RNA was eluted into RNase-free water.

RNA purified in this way was analysed by agarose gel to establish purity and quality. The gel is shown in figure 2.

Microarray analysis

Microarray analysis was undertaken as a commercial service by the University of Newcastle-upon-Tyne. In this study, the 2 RNA samples (1x butyrate + 1x glucose) from the 2 experimental conditions (butyrate + glucose) were sent to the Institute for Human Genetics at the University of Newcastle-upon-Tyne for microarray analysis. This was performed on a 12 k Affymetrix *Homo sapiens* gene chip. Genes altered in expression by more than 2 fold on the microarray are listed in table 1.

Claims

1. A method to screen for nucleic acid molecules which show altered expression in an isolated first cell sample comprising comparing the gene expression profiles between said first cell sample with a second reference cell sample wherein said first cell sample has been grown in the presence of the carbon source butyrate, or a related carbon source from which butyrate is derived, either directly or indirectly, and comparing said expression profile with the expression profile in said second reference cell sample which has not been grown in the presence of butyrate, or said related carbon source.

2. A method according to Claim 1 wherein said screen for nucleic acid molecules comprises the steps of:

i) providing
   a) a cell growth preparation comprising a first cell sample derived from at least one region of the colon; cell growth media; and a carbon source wherein said carbon source is butyrate; and
   b) a cell growth preparation comprising a second cell sample derived from an equivalent region of the colon; cell growth media; and a carbon source which is not butyrate;

ii) extracting nucleic acid from said first and second cell samples; and

iii) comparing the gene expression profile in said first cell sample with the gene expression profile in said second cell sample.

3. A method according to Claim 1 or 2 wherein said first and second cell samples are derived from the ascending colon.

4. A method according to Claim 1 or 2 wherein said first and second cell samples are derived from the transverse colon.

5. A method according to Claim 1 or 2 wherein said first and second samples are derived from the descending colon.

6. A method according to Claim 1 or 2 wherein said first and second samples are derived from the sigmoid region of the colon.

7. A method according to Claim 6 wherein said cell samples are derived from the rectal region of the colon.

8. A method according to any of Claims 1-7 wherein said first and second cell samples comprise epithelial cells.

9. A method according to any of Claims 1-8 wherein said carbon source which is not butyrate is glucose.

10. A method according to any of Claims 1-9 wherein said nucleic acid molecule which shows altered expression is selected from the group as represented by the nucleic acid sequences as shown in Table 1, or nucleic acid molecules which hybridise to the sequences presented in Table 1.

11. A method for the detection of at least one nucleic acid molecule associated with the initiation and/or progression of colorectal cancer, in an animal, comprising the steps of:

i) providing a biological sample comprising at least one cell to be tested;

ii) contacting said sample with a ligand which binds at least one nucleic acid molecule as represented by the nucleic acid sequence selected from the group consisting of:

a) a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1;

b) a nucleic acid molecule which hybridises to nucleic acid molecules as defined in (a);
c) a nucleic acid molecule that is degenerate because of the genetic code to the nucleic acid molecule represented in (a) and (b); and

iii) detecting the presence of at least one nucleic acid molecule in said sample.

12. A method according to Claim 11 wherein said colorectal cancer is adenocarcinoma.

13. A method according to Claim 11 or 12 wherein said ligand is a nucleic acid molecule adapted to anneal to said nucleic acid molecule which is associated with colorectal cancer.

14. A method according to Claim 13 wherein said method is a polymerase chain reaction method.

15. A method for the detection of at least one polypeptide associated with the initiation and/or progression of colorectal cancer, in an animal, comprising the steps of:

i) providing a biological sample comprising at least one cell to be tested;

ii) contacting said sample with at least one ligand which ligand specifically binds at least one polypeptide encoded by a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1, or a variant polypeptide comprising an amino acid sequence which varies by the addition, deletion or substitution of at least one amino acid residue of the amino acid sequence shown in Table 1; and

iii) detecting the presence of at least one polypeptide in said sample.

16 A method according to any of Claims 11-15 wherein said animal is human.

17. A method according to Claim 15 or 16 wherein said ligand is an antibody.

18. A method according to Claim 17 wherein said antibody is a monoclonal antibody, or at least the effective binding part thereof.

19. The use of at least one polypeptide, or variant sequence thereof, encoded by a nucleic acid molecule(s) as represented by the nucleic acid sequence as shown in Table 1, as a target for the screening of agents which modulate the activity of said polypeptide.

20. A method to screen for agents which modulate the activity of at least one polypeptide encoded by a gene associated with the initiation and/or progression of colorectal cancer comprising the steps of:

i) forming a preparation comprising at least one polypeptide wherein said polypeptide is encoded by a nucleic acid sequence as shown in Table 1, or a variant polypeptide comprising an amino acid sequence which varies by the addition, deletion or substitution of at least one amino acid residue of the amino acid sequence shown in Table 1 and at least one agent to be tested; and

ii) determining the activity of said agent with respect to activity of said polypeptide.

21. A method according to Claim 20 wherein said polypeptide is expressed by a cell wherein said cell is transformed or transfected with said nucleic acid molecule.

22. A method according to Claim 21 wherein said nucleic acid molecule is part of a vector adapted for recombinant expression of said nucleic acid molecule.

23. A method according to Claim 22 wherein said vector is provided with a promoter which enables the expression of said nucleic acid molecule to be regulated.

24. A method according to any of Claims 21-23 wherein said cell is derived from the colon.

25. A method according to Claim 24 wherein said cell is an epithelial cell.

26. A method according to any of Claims 20-25 wherein said agent is an antibody.

27. A method according to Claim 26 wherein said antibody is a monoclonal antibody or modified monoclonal antibody, or at least the effective binding part thereof.

28. A method according to Claim 27 wherein said binding part is a Fab fragment.

29. A method according to Claim 28 wherein said antibody is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; antibodies comprising CDR3 regions, and single chain antibody variable regions.

30. A method according to Claim 26 wherein said antibody is a humanised.

31. A method according to Claim 26 wherein said antibody is a chimeric antibody.

32. A method according to any of Claims 20-25 wherein said agent is a polypeptide.

33. A method according to any of Claims 20-25 wherein said agent is a peptide.

34. A method according to any of Claims 20-25 wherein said agent is nucleic acid molecule.

35. A method according to Claim 34 wherein said nucleic acid molecule is an aptamer.

36. A method according to Claim 34 wherein said nucleic acid is an inhibitory RNA molecule.

37. A method according to Claim 36 wherein said inhibitory RNA is encoded by a transcription cassette comprising a nucleic acid molecule, or part thereof, selected from the group consisting of:

i) a nucleic acid molecule as represented by the nucleic acid sequence as shown in Table 1;

ii) a nucleic acid molecule which hybridises to the sequence in (i); or

iii) a nucleic acid molecule which is degenerate because of the genetic code to the sequences defined in (i) and (ii) above; wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

38. A method according to Claim 37 wherein said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

39. A method according to Claim 37 wherein said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

40. A method according to Claim 34 wherein said nucleic acid molecule is an antisense nucleic acid molecule.

41. An antibody, or effective binding part thereof, identified by the method according to any of Claims 26-31 for use as a pharmaceutical.

42. A polypeptide identified by the method according to Claim 32 for use as a pharmaceutical.

43. A peptide identified by the method according to Claim 33 for use as a pharmaceutical.

44. A nucleic acid molecule identified by the method according Claim 34 for use as a pharmaceutical.

45. Use according to Claim 44 wherein said nucleic acid molecule is an aptamer.

46. Use according to Claim 44 wherein said nucleic acid molecule is an inhibitory RNA.

47. Use according to Claim 44 wherein said nucleic acid molecule is an antisense nucleic acid molecule.

48. Use according to any of Claims 41-47 wherein said pharmaceutical further comprises a a diluent, carrier or excipient.

Abstract

We describe a method for the identification of genes which show regulated expression in response to carbon source utilisation, typically genes associated with the initiation and/or promotion of cell transformation from a non-cancerous to a cancerous phenotype, typically of cells found in the colon; the use of these genes in diagnostic assays and as targets for the development of chemotherapeutic drugs and agents identified by said assay.

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agtcattaaa ggagctgttt cctgggtaaa aaaaaaaaa a
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3 Homo sapiens Apo-2 ligand mRNA, complete cds.

/translation="MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQM
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EKQQNISPLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSF
LSNLHLRNGELVIHEKGFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILL
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3'UTR 937..1042

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Sequence 1042 BP; 348 A; 208 C; 232 G; 254 T; 0 other;
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agcctgggac agacctgcgt gctgatcgtg atcttcacag tgctcctgca gtctctctgt     180
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aaaagtggca ttgcttgttt cttaaaagaa gatgacagtt attgggaccc caatgacgaa     300
gagagtatga acagcccctg ctggcaagtc aagtggcaac tccgtcagct cgttagaaag     360
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E    Homo sapiens mRNA for annexin A13 (ANXA13 gene), isoform b

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T                        AIKEAYQRLFDRSLESDVKGDTSGNLKKILVSLLQANRNEGDDVDKDLAGQDAKDLYDA
T                        GEGRWGTDELAFNEVLAKRSYKQLRATFQAYQILIGKDIEEAIEEETSGDLQKAYLTLV
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     actatgcatt tgtaatggca catttagaag gatatgcatc acacaagtaa ggtacaggaa      1500
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)E Homo sapiens serine protease inhibitor, Kazal type 1, mRNA (cDNA clone

Sequence 362 BP; 121 A; 74 C; 75 G; 92 T; 0 other;

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cttaatggat gcaccaagat atatgaccct gtctgtggga ctgatggaaa tacttatccc      180
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a

'E Homo sapiens B cell linker protein BLNK mRNA, alternatively spliced,
'E complete cds.

'T /translation="MDKLNKITVPASQKLRQLQKMVHDIKNNEGGIMNKIKKLKVKAPP
'T SVPRRDYASESPADEEEQWSDDFDSDYENPDEHSDSEMYVMPAEENADDSYEPPPVEQE
'T TRPVHPALPFARGEYIDNRSSQRHSPPFSKTLPSKPSWPSEKARLTSTLPALTALQKPQ
'T VPPKPKGLLEDEADYVVPVEDNDENYIHPTESSSPPPEKAPMVNRSTKPNSSTPASPPG
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Homo sapiens cDNA FLJ12768 fis, clone NT2RP2001576, weakly similar to HYPOTHETICAL 62.2 KD PROTEIN C4G8.12C IN CHROMOSOME I.

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Sequence 2687 BP; 454 A; 883 C; 733 G; 617 T; 0 other;
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Homo sapiens glycine amidinotransferase (L-arginine:glycine amidinotransferase), mRNA (cDNA clone MGC:1744 IMAGE:3010128), complete

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Sequence 2342 BP; 690 A; 490 C; 480 G; 682 T; 0 other;
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DE   POLIOVIRUS RECEPTOR PRECURSOR.

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     agggaactcg agagcagcct ccatgggcac acaggagggc tggtgcctgc tgctctgcct       180
     ggctctatct ggagcagcag aaaccaagcc ccacccagca gaggggcagt ggcgggcagt       240
     ggacgtggtc ctagactgct tcctggcgaa ggacggtgcg caccgtggag ctctcgccag       300
     cagtgaggac agggcaaggg cctcccttgt gctgaagcag gtgccagtgc tggacgatgg       360
     ctccctggag gacttcaccg atttccaagg gggcacactg gcccaagatg acccacctat       420
     tatctttgag gcctcagtgg acctggtcca gattccccag gccgaggcct tgctccatgc       480
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     gaccactgtt aagacagcag cttggttcat ggccaacgtg caggtctctg gacggggacc       600
     tagcatctcc ttggtgatga agactcccag ggtcgccaag aatgaggcgc tctggcaccc       660
     gacgctgaac ttgccactga gcccccaggg gactgtgcga actgcagtgg agttccaggt       720
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     gcagatcatc cagctcaaca tccaagcttc ccctaaagta cgactgagct tggcaaacga      1080
     agctctgctg cccaccctca tctgcgacat tgctggctat taccctctgg atgtggtggt      1140
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     ctcccatctc catgaagacc gcacagcgcg tgtaagccag cccagctgac ctaaagcgac      1560
     atgagactac tagaaagaaa cgacaccctt ccccaagccc ccacagctac tccaacccaa      1620
     acaacaacca agccagttta atggtaggaa tttgtatttt ttgcctttgt tcagaataca      1680
tgacattggt aaat
```

DE Homo sapiens leucine aminopeptidase 3, mRNA (cDNA clone IMAGE:2821948), partial cds.

```
/translation="LAVRRFGSRSLSTADMTKGLVLGIYSKEKEDDVPQFTSAGENFDK
LLAGKLRETLNISGPPLKAGKTRTFYGLHQDFPSVVLVGLGKKAAGIDEQENWHEGKEN
IRAAVAAGCRQIQDLELSSVEVDPCGDAQAAAEGAVLGLYEYDDLKQKKKMAVSAKLYG
SGDQEAWQKGVLFASGQNLARQLMETPANEMTPTRFAEIIEKNLKSASSKTEVHIRPKS
WIEEQAMGSFLSVAKGSDEPPVFLEIHYKGSPNANEPPLVFVGKGITFDSGGISIKASA
NMDLMRADMGGAATICSAIVSAAKLNLPINIIGLAPLCENMPSGKANKPGDVVRAKNGK
TIQVDNTDAEGRLILADALCYAHTFNPKVILNAATLTGAMDVALGSGATGVFTNSSWLW
NKLFEASIETGDRVWRMPLFEHYTRQVVDCQLADVNNIGKYRSAGACTAAAFLKEFVTH
PKWAHLDIAGVMTNKDEVPYLRKGMTGRPTRTLIEFLLRFSQDNA"
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Sequence 1938 BP; 603 A; 386 C; 470 G; 479 T; 0 other;
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gagagaattt tgataaattg ttagctggaa agctgagaga gactttgaac atatctggac 180
cacctctgaa ggcagggaag actcgaacct tttatggtct gcatcaggac ttccccagcg 240
tggtgctagt tggcctcggc aaaaaggcag ctggaatcga cgaacaggaa aactggcatg 300
aaggcaaaga aaacatcaga gctgctgttg cagcggggtg caggcagatt caagacctgg 360
agctctcgtc tgtggaggtg gatccctgtg gagacgctca ggctgctgcg gagggagcgg 420
tgcttggtct ctatgaatac gatgacctaa agcaaaaaaa gaagatggct gtgtcggcaa 480
agctctatgg aagtggggat caggaggcct ggcagaaagg agtcctgttt gcttctgggc 540
agaacttggc acgccaattg atggagacgc cagccaatga gatgacgcca accagatttg 600
ccgaaattat tgagaagaat ctcaaaagtg ctagtagtaa aaccgaggtc catatcagac 660
ccaagtcttg gattgaggaa caggcaatgg gatcattcct cagtgtggcc aaaggatctg 720
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tgattaaaat ttttagaact tcctaatcac ttttcagagt atatgttttt cattgagaag 1800
caaaattgta actcagattt gtgatgctag gaacatgagc aaactgaaaa ttactatgca 1860
cttgtcagaa acaataaatg caacttgttg tgctcaaaaa aaaaaaaaa aaaaaaaaa 1920
aaaaaaaaa aaaaaaa
```

'E Homo sapiens mRNA for protein phosphatase 4 regulatory subunit 2 (PPP4R2
'E gene)

'T /translation="MCQAPCWRAGGSGLGRCSLCRSCSLARFPRLPSFPPPGRLRAGVC
'T AREGEGVGGVGGGVPVPKRPAEGGGGCEGLREAMDVERLQEALKDFEKRGKKEVCPVLD
'T QFLCHVAKTGETMIQWSQFKGYFIFKLEKVMDDFRTSAPEPRGPPNPNVEYIPFDEMKE
'T RILKIVTGFNGIPFTIQRLCELLTDPRRNYTGTDKFLRGVEKNVMVVSCVYPSSERNNS
'T NSLNRMNGVMFPGNAPSYTERSNINGPGTPRPRNRPKVSLSAPMTTNGWPESTDSKEAN
'T LQQNEEKTHSDSSTSESEVSSVSPLRNKHPDEDAVEAEGHEVKRLRFDKEGEVRETASQ
'T TTSSEISSVMVGETEASSSSQDKDKDSRCTRQHCTEEDEEEDEEEEEESFMTSREMIPE
'T RKNQEKESDDALTVNEETSEENNQMEESDVSQAEKDLLHSEGSENEGPESKWFF"
X
Q Sequence 2049 BP; 651 A; 409 C; 506 G; 483 T; 0 other;

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cctcaccatg gatacgcttg tgatcaaggg cctggtctcc cctcaagaca cggtcacaga 180
tcagaggcca caccatccta gcagtggagc agtaccagct gggacagggt ccttctgtga 240
cacctgctgc atcaccaggc tgggtgaacg gacacaattg ccagaactca cagaatagaa 300
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gagaggctcc aggaggcgct gaaagatttt gagaagaggg ggaaaaagga agtttgtcct 720
gtcctggatc agtttctttg tcatgtagcc aagactggag aaacaatgat tcagtggtcc 780
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gctcctgagc caagaggtcc tcccaaccct aatgtcgaat atattccctt tgatgaaatg 900
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ggagtagaaa agaacgtgat ggttgttagc tgtgtttatc cttcttcaga gagaaacaat 1080
tccaatagtt taaatcgaat gaatggtgtg atgtttcctg gaaatgcacc aagctatact 1140
gagaggtcta atataaatgg gcctgggaca cccaggccac gtaatcgacc aaaggtttct 1200
ctgtcagccc ccatgacaac aaatgggtgg cctgagagca cagacagcaa agaggcaaat 1260
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agtaggaacc aattcccagt aaaactggaa agaatctttc cagaatcatc ccatggataa 1860
tgatgacgaa gccacagaag tcaccgatga accactggaa caagactatt tagaaacatt 1920
tacatgcagt attttacaca cagttctggt tttaacactg tataaactt ttatgtaaaa 1980
aagtgcacct ttagttttac aagtaaagca ggttgtaaaa taaagtactt tatggataat 2040
tcctgaaag
```

Human mRNA for (2'-5') oligo A synthetase E (1,6 kb RNA)

/translation="MMDLRNTPAKSLDKFIEDYLLPDTCFRMQIDHAIDIICGFLKERC
FRGSSYPVCVSKVVKGGSSGKGTTLRGRSDADLVVFLSPLTTFQDQLNRRGEFIQEIRR
QLEACQRERALSVKFEVQAPRWGNPRALSFVLSSLQLGEGVEFDVLPAFDALGQLTGSY
KPNPQIYVKLIEECTDLQKEGEFSTCFTELQRDFLKQRPTKLKSLIRLVKHWYQNCKKK
LGKLPPQYALELLTVYAWERGSMKTHFNTAQGFRTVLELVINYQQLCIYWTKYYDFKNP
IIEKYLRRQLTKPRPVILDPADPTGNLGGGDPKGWRQLAQEAEAWLNYPCFKNWDGSPV
SSWILLVRPPASSLPFIPAPLHEA"

Sequence 1322 BP; 334 A; 353 C; 320 G; 315 T; 0 other;

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gatcagttaa atcgccgggg agagttcatc caggaaatta ggagacagct ggaagcctgt      360
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cctgcttcct ccctgccatt catccctgcc cctctccatg aagcttgaga catatagctg     1140
gagaccattc tttccaaaga acttacctct tgccaaaggc catttatatt catatagtga     1200
caggctgtgc tccatatttt acagtcattt tggtcacaat cgagggtttc tggaattttc     1260
acatcccttg tccagaattc attcccctaa gagtaataat aaataatctc taacaccaaa     1320
a
```

ΙE   Homo sapiens A-kinase anchoring protein 18 beta mRNA, complete cds.

'T   /translation="MGQLCCFPFSRDEGKISELESSSSAVLQRYSKDIPSWSSGEKNGG
'T   EPDDAELVRLSKRLVENAVLKAVQQYLEETQNKNKPGEGSSVKTEAADQNGNDNENNRK
'T   "
:X
:Q   Sequence 463 BP; 139 A; 106 C; 132 G; 86 T; 0 other;

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     tgaaggaaaa atcagtgagt tggaaagctc gtcctctgca gtcctacaaa gatacagcaa       180
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     agtaaggctc agtaagaggc tggtggagaa cgcggtgctc aaggctgtcc agcagtatct       300
     ggaggaaaca cagaataaaa acaagccggg ggaggggagc tctgtgaaaa ccgaagcagc       360
     tgatcagaat ggcaatgaca atgagaacaa caggaaatga gcccggaacg caggccccca       420
tgtctctgtg caaagcctcc ctgcttccct ctgctgagtc tag
```

Homo sapiens peptidyl prolyl isomerase H (cyclophilin H), mRNA (cDNA clone

/translation="MAVANSSPVNPVVFFDVSIGGQEVGRMKIELFADVVPKTAENFRQ
FCTGEFRKDGVPIGYKGSTFHRVIKDFMIQGGDFVNGDGTGVASIYRGPFADENFKLRH
SAPGLLSMANSGPSTNGCQFFITCSKCDWLDGKHVVFGKIIDGLLVMRKIENVPTGPNN
KPKLPVVISQCGEM"

Sequence 765 BP; 199 A; 156 C; 200 G; 210 T; 0 other;

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aactgtaa ataaagtttt tttgtatgcg taaaaaaaaa aaaaa
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E   Homo sapiens mRNA; cDNA DKFZp564C0362 (from clone DKFZp564C0362); complete
E   cds

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T   IRWEKNITLGEPPGFLHSWWCVFWDLYCAAPERRETCEHSSEAKAFHDYSAAAAPSPVL
T   GNIPPGDGMPVGPVPPGFFQPFMSPRYPGGPRPPLRIPNQALGGVPGSQPLLPRGMDPT
T   RQQGHPNMGGPMQRMTPPRGMVPLGPQNYGGAMRPPLNALGGPGMPGMNMGPGGGRPWP
T   NPTNANSIPYSSASPGNYVGPPGGGGPPGTPIMPSPADSTNSGDNMYTLMNAVPPGPNR
T   PNFPMGPGSDGPMGGLGGMESHHMNGSLGSGDMDSISKNSPNNMSLSNQPGTPRDDGEM
T   GGNFLNPFQSESYSPSMTMSV"
T   polyA signal   1685..1690
T   polyA site   1711
X
Q   Sequence 1731 BP; 513 A; 385 C; 392 G; 441 T; 0 other;
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     agttagcact ctacgtatat gaatatctgc tccatgtagg agctcagaaa tcagctcaaa   180
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     ggggaaattt cttaaatcct ttttcagagtg agagttactc ccctagcatg acaatgagcg   1140
     tgtgatccat taccaagtct cctcatgaaa accacagtga gtcagccctt cacagaacta   1200
     ctacggaaga aaattattca tcacagtgta cagttaaaca aaggaatctc agtcacacca   1260
     aaccaacctt ttcatttcct gctctctccc ctcttttgtg aagaaagcgg gtccagatgt   1320
     gattcaaaca actgtacgga gtggcatatt agaattgccc taaactgaac tgcaaataat   1380
     tatgtgtgta tgtatatgtg tgggaaagag aatgtactgt atatgtgtat gttatacaga   1440
     catatacaca tacatacatt gacccacagg acattgtaaa atattatcac atgacatctt   1500
     aagtagaaat aagtagggac ttttattcca tcctttttt cacgtttaca ttttaattat   1560
     tacaagttgc tcctgccccc tccctgaact atttgtgct gtgtatatca ctgctttata   1620
     taagttattt tttaaggtga actcagatgt tatggttttg tatatgtctg caatcatgga   1680
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Human interferon-induced cellular resistance mediator protein (MxB) mRNA, complete cds.

/translation="MSKAHKPWPYRRRSQFSSRKYLKKEMNSFQQQPPPFGTVPPQMMF
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DSLRALGVEQDLALPAIAVIGDQSSGKSSVLEALSGVALPRGSGIVTRCPLVLKLKKQP
CEAWAGRISYRNTELELQDPGQVEKEIHKAQNVMAGNGRGISHELISLEITSPEVPDLT
IIDLPGITRVAVDNQPRDIGLQIKALIKKYIQRQQTINLVVVPCNVDIATTEALSMAHE
VDPEGDRTIGILTKPDLMDRGTEKSVMNVVRNLTYPLKKGYMIVKCRGQQEITNRLSLA
EATKKEITFFQTHPYFRVLLEEGSATVPRLAERLTTELIMHIQKSLPLLEGQIRESHQK
ATEELRRCGADIPSQEADKMFFLIEKIKMFNQDIEKLVEGEEVVRENETRLYNKIREDF
KNWVGILATNTQKVKNIIHEEVEKYEKQYRGKELLGFVNYKTFEIIVHQYIQQLVEPAL
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MVFCQDQIYSVVLKKVREEIFNPLGTPSQNMKLNSHFPSNESSVSSFTEIGIHLNAYFL
ETSKRLANQIPFIIQYFMLRENGDSLQKAMMQILQEKNRYSWLLQEQSETATKRRILKE
RIYRLTQARHALCQFSSKEIH"

Sequence 2961 BP; 826 A; 754 C; 721 G; 660 T; 0 other;
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U90547;
. 70, Last updated, Version 4)

Human Ro/SSA ribonucleoprotein homolog (RoRet) mRNA, complete cds.

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Sequence 2872 BP; 892 A; 584 C; 688 G; 708 T; 0 other;
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)E Homo sapiens histone 2, H2aa, mRNA (cDNA clone MGC:2238 IMAGE:3536984),
)E complete cds.

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:Q Sequence 567 BP; 136 A; 171 C; 168 G; 92 T; 0 other;

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Homo sapiens transcription factor ISGF-3 mRNA, complete cds.

transcription factor.

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Sequence 4003 BP; 1173 A; 812 C; 883 G; 1135 T; 0 other;

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attaaatata atatcgacac agtgctttcc gtggcactgc atacaatctg aggcctcctc 3900
tctcagtttt tatatagatg gcgagaacct aagtttcagt tgattttaca attgaaatga 3960
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Homo sapiens mRNA; cDNA DKFZp564K2478 (from clone DKFZp564K2478); complete

/translation="MSKAFGLLRQICQSILAESSQSPADLEEKKEEDSNMKREQPRERP
RAWDYPHGLVGLHNIGQTCCLNSLIQVFVMNVDFTRILKRITVPRGADEQRRSVPFQML
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LQALYTIRVKDSLICVDCAMESSRNSSMLTLPLSLFDVDSKPLKTLEDALHCFFQPREL
SSKSKCFCENCGKKTRGKQVLKLTHLPQTLTIHLMRFSIRNSQTRKICHSLYFPQSLDF
SQILPMKRESCDAEEQSGGQYELFAVIAHVGMADSGHYCVYIRNAVDGKWFCFNDSNIC
LVSWEDIQCTYGNPNYHWQETAYLLVYMKMEC"

Sequence 1874 BP; 481 A; 436 C; 489 G; 468 T; 0 other;

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ccaggcagct gcggcctggg ggttttggag tgatcacgaa tgagcaaggc gtttgggctc    180
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aaaaaaaaaa aaaa                                                     1874
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```
DE   Homo sapiens cDNA FLJ20073 fis, clone COL02320.

FT                   /translation="MGTYSTILIKTEVIECGNYCGVRIIHSLIAEFSLEELKKSYHLNK
FT                   SQIMLDMLTENLFFDTGMGKSKFLQDMHTLLLTRHRDEHEGETGNWFSPFIEALHKDEG
FT                   NEAVEAVLLESIHRFNPNAFICQALARHFYIKKKDFGNALNWAKQAKIIEPDNSYISDT
FT                   LGQVYKSKIRWWIEENGGNGNISVDDLIALLDLAEHASSAFKESQQQSEDREYEVKERL
FT                   YPKSKRRYDTYNIAGYQGEIEVGLYTIQILQLIPFFDNKNELSKRYMVNFVSGSSDIPG
FT                   DPNNEYKLALKNYIPYLTKLKFSLKKSFDFFDEYFVLLKPRNNIKQNEEAKTRRKVAGY
FT                   FKKYVDIFCLLEESQNNTGLGSKFSEPLQVERCRRNLVALKADKFSGLLEYLIKSQEDA
FT                   ISTMKCIVNEYTFLLEQCTVKIQSKEKLNFILANIILSCIQPTSRLVKPVEKLKDQLRE
FT                   VLQPIGLTYQFSEPYFLASLLFWPENQQLDQHSEQMKEYAQALKNSFKGQYKHMHRTKQ
FT                   PIAYFFLGKGKRLERLVHKGKIDQCFKKTPDINSLWQSGDVWKEEKVQELLLRLQGRAE
FT                   NNCLYIEYGINEKITIPITPAFLGQLRSGRSIEKVSFYLGFPIGGPLAYDIEIV"
XX
SQ   Sequence 3401 BP; 1260 A; 588 C; 619 G; 934 T; 0 other;
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aagattccat ggttatgttt tttaaagagt tcatgccttt tagagataca tactaaagta 3360
tttgtaaata aatgacatga tctagaaaaa aaaaaaaaaa a 3401

E   Homo sapiens cDNA FLJ10913 fis, clone OVARC1000209, weakly similar to Oryza
E   sativa submergence induced protein 2A mRNA.

T   /translation="MVLAWYMDDAPGDPRQPHRPDPGRPVGLEQLRRLGVLYWKLDADK
T   YENDPELEKIRRERNYSWMDIITICKDKLPNYEEKIKMFYEEHLHLDDEIRYILDGSGY
T   FDVRDKEDQWIRIFMEKGDMVTLPAGIYHRFTVDEKNYTKAMRLFVGEPVWTAYNRPAD
T   HFEARGQYVKFLAQTA"
X
Q   Sequence 1628 BP; 440 A; 349 C; 389 G; 450 T; 0 other;

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Homo sapiens cDNA: FLJ22242 fis, clone HRC02528.

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LLCSHGHCCITESGPLLSFSNWPPSLVPHFLKSPVHCHQIKLSPARSPLSEKPPLTWKH
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Sequence 1300 BP; 268 A; 413 C; 227 G; 392 T; 0 other;
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DE   ta77f02.x2 NCI_CGAP_HSC2 Homo sapiens cDNA clone IMAGE:2050107 3' similar
DE   to gb:L19779 HISTONE H2A.1 (HUMAN);, mRNA sequence.

     tatacggctg cgagaagacg acagaagggg cacctgtgaa ctcaaaaggc tcttttcaga        60
     gccacccacg ttttcaaata aaagagttgt taatgctggc cactcccaaa aaaaaaaaaa       120
     aaaaaaaaaa agtcgtatcg a                                                 141
/
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H.sapiens centromere autoantigen C (CENPC) mRNA, complete cds.

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MSGKNDVDDEEVHGSSDDSKQSKVIPKNRIHHKLVLPSNTPNVRRTKRTRLKPLEYWRG
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Sequence 3132 BP; 1164 A; 542 C; 630 G; 796 T; 0 other;
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Homo sapiens transcription factor ISGF-3 mRNA, complete cds.

transcription factor.

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Homo sapiens ornithine decarboxylase (ODC1) mRNA, complete cds.

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)E Human 18S rRNA gene, complete.
(X
(W 18S ribosomal RNA; ribosomal RNA.

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Homo sapiens cell death regulator aven mRNA, complete cds.

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Sequence 1549 BP; 415 A; 349 C; 469 G; 314 T; 2 other;
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)E   Homo sapiens interferon, gamma-inducible protein 16, mRNA (cDNA clone
)E   MGC:9466 IMAGE:3914632), complete cds.

'T                       /translation="MGKKYKNIVLLKGLEVINDYHFRMVKSLLSNDLKLNLKMREEYDK
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DE Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa, mRNA
DE (cDNA clone MGC:3949 IMAGE:3606865), complete cds.

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Homo sapiens interferon induced transmembrane protein 1 (9-27), mRNA (cDNA clone MGC:5195 IMAGE:3464598), complete cds.

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DE   Homo sapiens transcription factor ISGF-3 mRNA, complete cds.

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XX
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DE   complete cds.
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Homo sapiens metalloprotease disintegrin cysteine-rich protein, secreted form mRNA, complete cds.

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)E Homo sapiens matrix metalloproteinase 7 (matrilysin, uterine), mRNA (cDNA
)E clone MGC:3913 IMAGE:3545760), complete cds.

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Homo sapiens cDNA FLJ10650 fis, clone NT2RP2005853.

fis (full insert sequence); oligo capping.

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E Homo sapiens transcription factor ISGF-3 mRNA, complete cds.
K
N transcription factor.

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)E Homo sapiens cDNA: FLJ21350 fis, clone COL02751.

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Homo sapiens IFI16b (IFI16b) mRNA, complete cds.

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Sequence 4151 BP; 1436 A; 806 C; 798 G; 1111 T; 0 other;

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Homo sapiens mRNA for STAT induced STAT inhibitor-2, complete cds.

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Sequence 704 BP; 198 A; 172 C; 174 G; 160 T; 0 other;

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:X
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E Homo sapiens interferon stimulated T-cell alpha chemoattractant precursor,
E mRNA, complete cds.

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Homo sapiens mRNA; cDNA DKFZp586J0323 (from clone DKFZp586J0323)

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Homo sapiens cDNA FLJ20637 fis, clone KAT03212.

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Sequence 2010 BP; 640 A; 415 C; 397 G; 558 T; 0 other;

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Homo sapiens sodium bicarbonate cotransporter (HNBC1) mRNA, complete cds.

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E Human BRCA1-associated RING domain protein (BARD1) mRNA, complete cds.

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Human mRNA for 56-KDa protein induced by interferon

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Sequence 1642 BP; 551 A; 318 C; 369 G; 404 T; 0 other;

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E sequence.

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Human interferon-induced cellular resistance mediator protein (MxA) mRNA, complete cds.

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Sequence 2651 BP; 732 A; 646 C; 704 G; 569 T; 0 other;

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Homo sapiens cDNA: FLJ21726 fis, clone COLF1088.

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E xw86e11.x1 NCI_CGAP_Pan1 Homo sapiens cDNA clone IMAGE:2834924 3', mRNA
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Human 71 kDa 2'5' oligoadenylate synthetase (p69 2-5A synthetase) mRNA, complete cds.

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Homo sapiens cDNA FLJ20035 fis, clone COL00213.

/translation="MSGRAGRRGQDLMGDVYFFDIPFPKIGKLIKSNVPELRGHFPLSI
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LDQEGNPMGFAGLVSHLHYHEPSNLVFVSFLVNGLFHDLCQPTRKGSKHFSQDVMEKLV
LVLAHLFGRRYFPPKFQDAHFEFYQSKVFLDDLPEDFSDALDEYNMKIMEDFTTFLRIV
SKLADMNQEYQLPLSKIKFTGKECEDSQLVSHLMSCKEGRVAISPFVCLSGNFDDDLLR
LETPNHVTLGTIGVNRSQAPVLLSQKFDNRGRKMSLNAYALDFYKHGSLIGLVQDNRMN
EGDAYYLLKDFALTIKSISVSLRELCENEDDNVVLAFEQLSTTFWEKLNKV"

Sequence 1906 BP; 626 A; 327 C; 359 G; 594 T; 0 other;

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cattccccaa aataggaaaa ctcataaaat ccaatgttcc tgagctgaga ggacacttcc    180
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```

```
E    Homo sapiens monocarboxylate transporter 2 (hMCT2) mRNA, complete cds.

T                        /translation="MPPMPSAPPVHPPPDGGWGWIVVGAAFISIGFSYAFPKAVTVFFK
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T                        FSSSVVQLYLTMGFITGLGLAFNLQPALTIIGKYFYRKRPMANGLAMAGSPVFLSSLAP
T                        FNQYLFNTFGWKGSFLILGSLLLNACVAGSLMRPLGPNQTTSKSKNKTGKTEDDSSPKK
T                        IKTKKSTWEKVNKYLDFSLFKHRGFLIYLSGNVIMFLGFFAPIIFLAPYAKDQGIDEYS
T                        AAFLLSVMAFVDMFARPSVGLIANSKYIRPRIQYFFSFAIMFNGVCHLLCPLAQDYTSL
T                        VLYAVFFGLGFGSVSSVLFETLMDLVGAPRFSSAVGLVTIVECGPVLLGPPLAGKLVDL
T                        TGEYKYMYMSCGAIVVAASVWLLIGNAINYRLLAKERKEENARQKSRESEPLSKSKHSE
T                        DVNVKVSNAQSVTSERETNI"
X
Q    Sequence 2104 BP; 602 A; 400 C; 447 G; 654 T; 1 other;
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     ttctgtagga ttaattgcaa actccaaata tattcgacct cgaattcagt acttcttcag      1080
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     agatagagtt gagagacaat taattatccc ctcttacaca caaacacaca tactcccaca      2040
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     aaaa                                                                  2104
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Homo sapiens interferon-induced protein 44, mRNA (cDNA clone MGC:24007

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IELRKSLLSALRTYEPYGSLVQQIRILLLGPIGAGKSSFFNSVRSVFQGHVTHQALVGT
NTTGISEKYRTYSIRDGKDGKYLPFILCDSLGLSEKEGGLCRDDIFYILNGNIRDRYQF
NPMESIKLNHHDYIDSPSLKDRIHCVAFVFDASSIQYFSSQMIVKIKRIRRELVNAGVV
HVALLTHVDSMDLITKGDLIEIERCEPVRSKLEEVQRKLGFALSDISVVSNYSSEWELD
PVKDVLILSALRRMLWAADDFLEDLPFEQIGNLREEIINCAQGKK"

```
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'E 601067066F1 NIH_MGC_10 Homo sapiens cDNA clone IMAGE:3453257 5', mRNA
'E sequence.

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```

Human glutamate receptor subunit (GluH1) mRNA, complete cds.

glutamate receptor subunit.

/translation="MQHIFAFFCTGFLGAVVGANFPNNIQIGGLFPNQQSQEHAAFRFA
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TAAEKNWQVTAVNILTTTEEGYRMLFQDLEKKKERLVVVDCESERLNAILGQIIKLEKN
GIGYHYILANLGFMDIDLNKFKESGANVTGFQLVNYTDTIPAKIMQQWKNSDARDHTRV
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LQQVRFEGLTGNVQFNEKGRRTNYTLHVIEMKHDGIRKIGYWNEDDKFVPAATDAQAGG
DNSSVQNRTYIVTTILEDPYVMLKKNANQFEGNDRYEGYCVELAAEIAKHVGYSYRLEI
VSDGKYGARDPDTKAWNGMVGELVYGRADVAVAPLTITLVREEVIDFSKPFMSLGISIM
IKKPQKSKPGVFSFLDPLAYEIWMCIVFAYIGVSVVLFLVSRFSPYEWHSEEFEEGRDQ
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AFLTVERMVSPIESAEDLAKQTEIAYGTLEAGSTKEFFRRSKIAVFEKMWTYMKSAEPS
VFVRTTEEGMIRVRKSKGKYAYLLESTMNEYIEQRKPCDTMKVGGNLDSKGYGIATPKG
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Sequence 3178 BP; 840 A; 741 C; 837 G; 760 T; 0 other;
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zn32e02.s1 Stratagene endothelial cell 937223 Homo sapiens cDNA clone IMAGE:549146 3', mRNA sequence.

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Homo sapiens mRNA for C11ORF25 gene

C11ORF25 gene.

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Homo sapiens isopentenyl-diphosphate delta isomerase, mRNA (cDNA clone

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XX
KW   prostaglandin endoperoxide synthase.

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c                                                                   901
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Homo sapiens mRNA for quinolinate phosphoribosyl transferase, complete cds.

nicotinate mononucleotide pyrophosphorylase; QPRTase;
quinolinate phosphoribosyl transferase.

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E Homo sapiens mRNA for cytochrome P-450 HFLa, complete cds.
X
W CYP3A6; cytochrome P-450; human fetal liver cytochrome P-450.

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T RRPFGPVGFMKNAISIAEDEEWKRIRSLLSPTFTSGKLKEMVPIIAQYGDVLVRNLRRE
T AETGKPVTLKHVFGAYSMDVITSTSFGVSIDSLNNPQDPFVENTKKLLRFNPLDPFVLS
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T KDSETHKALSDLELMAQSIIFIFAGYETTSSVLSFIIYELATHPDVQQKVQKEIDTVLP
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T VLHHDPKYWTEPEKFLPERFSKKNKDNIDPYIYTPFGSGPRNCIGMRFALVNMKLALVR
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Human mRNA for endothelin converting enzyme, complete cds.

endothelin converting enzyme.

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602386668F1 NIH_MGC_93 Homo sapiens cDNA clone IMAGE:4515521 5', mRNA sequence.

EST.

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Homo sapiens mRNA for Rev-ErbAalpha protein (hRev gene)

hRev gene; Rev-ErbAalpha; thyroid hormone receptor.

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yy35b09.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone IMAGE:273209 3', mRNA sequence.

```
ncagcatttt tcggcctctt tatttagaac ccggcggacg aggggccggg gcagtggtac    60
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ataagagaga cagcgaaanc cgaagagact gcaagctaga tgggcatgta tggcagctac   180
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aaccagatgt gggagatgga gagtgccttt gnantaacca ataaccgagc tagtgcgtgg   300
cagagcggtc cacgccttgg acataaatag aaaatataag ttagtataac tttaaaaact   360
ttttgtacaa atatacatgg tttttttant ttttccnttt ttttttcctt tttccttttt   420
ttgcactgag tttcagcaga gattaaacat tttatat                            457
```

DE Homo sapiens tumor rejection antigen (gp96) 1, mRNA (cDNA clone
DE IMAGE:3938823), complete cds.

FT /translation="MRALWVLGLCCVLLTFGSVRADDEVDVDGTVEEDLGKSREGSRTD
FT DEVVQREEEAIQLDGLNASQIRELREKSEKFAFQAEVNRMMKLIINSLYKNKEIFLREL
FT ISNASDALDKIRLISLTDENALSGNEELTVKIKCDKEKNLLHVTDTGVGMTREELVKNL
FT GTIAKSGTSEFLNKMTEAQEDGQSTSELIGQFGVGFYSAFLVADKVIVTSKHNNDTQHI
FT WESDSNEFSVIADPRGNTLGRGTTITLVLKEEASDYLELDTIKNLVKKYSQFINFPIYV
FT WSSKTETVEEPMEEEEAAKEEKEESDDEAAARRR"

```
gaggatccga acccaggggt ggggggtgga ggcggctcct gcgatcgaag gggacttgag    60
actcaccggc cgcacgccat gagggccctg tgggtgctgg gcctctgctg cgtcctgctg   120
accttcgggt cggtcagagc tgacgatgaa gttgatgtgg atggtacagt agaagaggat   180
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taaaatcttg tcatgtgtat aaaaataaaa aagatcccaa ataaaaaaaa aaaaaaaaaa  1380
a                                                                  1381
```

Homo sapiens tumor suppressor deleted in oral cancer-related 1, mRNA (cDNA clone MGC:3779 IMAGE:3659410), complete cds.

/translation="MSYKPIAPAPSSTPGSSTPGPGTPVPTGSVPSPSGSVPGAGAPFR
PLFNDFGPPSMGYVQAMKPPGAQGSQSTYTDLLSVIEEMGKEIRPTYAGSKSAMERLKR
GIIHARALVRECLAETERNART"

```
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cgcgcccggg aagacccctc ggcgcgaacc ggcagcccag ccccgggtcc cggttcccaa    180
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cggaaggcgg aggccaatcg gcggcggttg cgacctgctg gggcaggtct cggccaataa    360
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gagttgggga tgtcctacaa acccatcgcc cctgctccca gcagcacccc tggctccagc    600
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tcagcccttc ccagcacttc tcccactttg tcccgagcct ccttctcccc cagcaggggc   1260
acaggcctgg cacctccctg ccttgtgtcc tgagccatag tgactctttt atctgtgtgt   1320
cttttgctaa atatgccctt tttatattaa taaaagatga tttggagttg tgctctcaaa   1380
aaaaaaaaaa aaaaaa                                                    1397
```

E Homo sapiens TNFR-related death receptor-6 (DR6) mRNA, complete cds.

T /translation="MGTSPSSSTALASCSRIARRATATMIAGSLLLLGFLSTTTAQPEQ
T KASNLIGTYRHVDRATGQVLTCDKCPAGTYVSEHCTNTSLRVCSSCPVGTFTRHENGIE
T KCHDCSQPCPWPMIEKLPCAALTDRECTCPPGMFQSNATCAPHTVCPVGWGVRKKGTET
T EDVRCKQCARGTFSDVPSSVMKCKAYTDCLSQNLVVIKPGTKETDNVCGTLPSFSSSTS
T PSPGTAIFPRPEHMETHEVPSSTYVPKGMNSTESNSSASVRPKVLSSIQEGTVPDNTSS
T ARGKEDVNKTLPNLQVVNHQQGPHHRHILKLLPSMEATGGEKSSTPIKGPKRGHPRQNL
T HKHFDINEHLPWMIVLFLLLVLVVIVVCSIRKSSRTLKKGPRQDPSAIVEKAGLKKSMT
T PTQNREKWIYYCNGHGIDILKLVAAQVGSQWKDIYQFLCNASEREVAAFSNGYTADHER
T AYAALQHWTIRGPEASLAQLISALRQHRRNDVVEKIRGLMEDTTQLETDKLALPMSPSP
T LSPSPIPSPNAKLENSALLTVEPSPQDKNKGFFVDESEPLLRCDSTSSGSSALSRNGSF
T ITKEKKDTVLRQVRLDPCDLQPIFDDMLHFLNPEELRVIEEIPQAEDKLDRLFEIIGVK
T SQEASQTLLDSVYSHLPDLL"
K

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gctgaggaca aactagaccg gctattcgaa attattggag tcaagagcca ggaagccagc 1920
cagaccctcc tggactctgt ttatagccat cttcctgacc tgctgtag 1968

601848574F1 NIH_MGC_55 Homo sapiens cDNA clone IMAGE:4079202 5', mRNA sequence.

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gcttcaccgg gcataaggcg gacaacggtc ttaggcaaac tcgggtcctc gaaac         775
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DE   Homo sapiens clone PP1722 unknown mRNA.

FT   /translation="MQYLAATAASGAFVPPPSAQEIPVVSAPAPAPIHNQFPAENQPAN
FT   QNAAPQVVVNPGANQNLRMNAQGGPIVEEDDEINRDWLDWTYSAATFSVFLSILYFYSS
FT   LSRFLMVMGATVVMYLHHVGWFPFRPRPVQNFPNDGPPPDVVNQDPNNNLQEGTDPETE
FT   DPNHLPPDRDVLDGEQTSPSFMSTAWLVFKTFFASLLPEGPPAIAN"
XX
SQ   Sequence 2217 BP; 612 A; 460 C; 463 G; 682 T; 0 other;

```
     gctgtgtggc ccaggctttt ctcaaactcc tgagggcaag cgatcctccc acctcagcct        60
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     acacgaaacc atttattttt catttcacaa tgtttattca catatatggt attagtattc       180
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     tagcatctat gtattgaaaa ttatgttgaa taaatgtcag actatttttt acataacgtt       600
     gcttctgttt aattttgtca cgttcagagg tggggggtag gagatgtaag cccttgacag       660
     caaaataatt ccttttgctt gatttcagac agttgcatca gctcctttgt tctgtgttca       720
     tgttacactt atttaggtgg ctgaatccac agaggagcct gctggttcta atcggggaca       780
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     gatatatgca cgacagtact acatgcaata tttagcagcc actgctgcat caggggcttt      1020
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     agaagatgat gaaataaatc gagattggtt ggattggacc tattcagcag ctacattttc      1260
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     ggccaccgtt gttatgtacc tgcatcacgt tgggtggttt ccatttagac cgaggccggt      1380
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     gctgggtttt ctgttcaata aagttttact atgaatgaca aaaaaaaaaa aaaaaaa         2160
```

Homo sapiens hypothetical protein FLJ11259, mRNA (cDNA clone MGC:8787 IMAGE:3925141), complete cds.

/translation="MGIVANFQELAVPVVHDGGALLAFVCGVVYTLLQSIISYKSCPQW
NSLSTCHIRMVISAVSCAAVIPMIVCASLISITKLEWNPREKDYVYHVVSAICEWTVAF
GFIFYFLTFIQDFQSVTLRISTEINGDI"

Sequence 2388 BP; 725 A; 460 C; 523 G; 680 T; 0 other;

```
gcaaaatcaa acctgctatt tcagcactcc tgtttttaac ttggtgtctt tagtgcttgg       60
attggtggga tgtttcggaa tgggcattgt cgccaatttt caggagttag ctgtgccagt      120
ggttcatgac gggggcgctc ttttggcctt tgtctgtggt gtcgtgtaca cgctcctaca      180
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gatggtcatc tctgccgttt cttgcgcagc tgtcatcccc atgattgtct gtgcttcact      300
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tgctaatcca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa                   2388
```

'E tq65c10.x1 NCI_CGAP_Lu19 Homo sapiens cDNA clone IMAGE:2213682 3' similar
'E to SW:ENPL_HUMAN P14625 ENDOPLASMIN PRECURSOR ;, mRNA sequence.

```
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Homo sapiens phosphoserine aminotransferase (PSA) mRNA, complete cds.

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FIPDVKGAVLVCDMSSNFLSKPVDVSKFGVIFAGAQKNVGSAGVTVVIVRDDLLGFALR
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```

'E Homo sapiens cDNA clone:ADBAPE04, 5'end, expressed in human adrenal gland.

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wd68f02.x1 NCI_CGAP_Lu24 Homo sapiens cDNA clone IMAGE:2336763 3', mRNA sequence.

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E H.sapiens LU gene for Lutheran blood group glycoprotein.
X
W Lutheran blood group glycoprotein.

```
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r                    DSAQEIATCNSRNGNPAPKITWYRNGQRLEVPVEMNPEGYMTSRTVREASGLLSLTSTL
r                    YLRLRKDDRDASFHCAAHYSLPEGRHGRLDSPTFHLTLHYPTEHVQFWVGSPSTPAGWV
r                    REGDTVQLLCRGDGSPSPEYTLFRLQDEQEEVLNVNLEGNLTLEGVTRGQSGTYGCRVE
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tc                                                                   2400
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Homo sapiens mRNA for calmegin, complete cds.

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wx78h04.x1 NCI_CGAP_Ov38 Homo sapiens cDNA clone IMAGE:2549815 3', mRNA sequence.

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E Human CD9 antigen mRNA, complete cds.

X

W CD9 antigen.

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Γ                    AAAIWGYSHKDEVIKEVQEFYKDTYNKLKTKDEPQRETLKAIHYALNCCGLAGGVEQFI
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Γ                    RNREMV"
K
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Homo sapiens cDNA clone:HEMBA1001328, 3' end, expressed in whole embryo, mainly head.

3'-end sequence (3'-EST); EST (expressed sequence tag); oligo capping.

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E Homo sapiens 7-dehydrocholesterol reductase, mRNA (cDNA clone MGC:1760
E IMAGE:3507516), complete cds.

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r LLLGLVGYYIFRVANHQKDLFRRTDGRCLIWGRKPKVIECSYTSADGQRHHSKLLVSGF
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Homo sapiens squalene epoxidase (ERG1) mRNA, complete cds.

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E Homo sapiens keratin 23 (histone deacetylase inducible), transcript variant
E 1, mRNA (cDNA clone MGC:26158 IMAGE:4838347), complete cds.

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/

Homo sapiens translocon-associated protein gamma subunit mRNA, complete cds.

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'/

Homo sapiens malic enzyme 1, NADP(+)-dependent, cytosolic, mRNA (cDNA clone MGC:39115 IMAGE:4870714), complete cds.

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E Homo sapiens livin inhibitor-of-apotosis (LIVIN) mRNA, complete cds.

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Homo sapiens drebrin 1, transcript variant 1, mRNA (cDNA clone MGC:1517
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Homo sapiens MDS019 (MDS019) mRNA, complete cds.

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DE   mitochondrial protein, complete cds.

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Human global transcription activator homologous sequence mRNA, complete cds.

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Homo sapiens mRNA for KIAA0895 protein, partial cds.

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Homo sapiens NUCB2 protein (NUCB2) mRNA, complete cds.

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3 Homo sapiens glucose-6-phosphate dehydrogenase, mRNA (cDNA clone MGC:8534
3 IMAGE:2822640), complete cds.

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Homo sapiens zinc finger protein 165 (Zpf165) mRNA, complete cds.

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T    "National Institutes of Health, Mammalian Gene Collection (MGC)
T    http://www.ncbi.nlm.nih.gov/MGC/";
L    Unpublished.
K
R    RZPD; IMAGp998O2410141.
R    UNILIB; 8584; 8584.
K
C    Contact: Robert Strausberg, Ph.D.
C    Tel: (301) 496-1550
C    Email: Robert_Strausberg@nih.gov
C    Tissue Procurement: ATCC
C    cDNA Library Preparation: Life Technologies, Inc.
C    cDNA Library Arrayed by: The I.M.A.G.E. Consortium (LLNL)
C    DNA Sequencing by: Incyte Genomics, Inc.
C    Clone distribution: MGC clone distribution information can be
C    found through the I.M.A.G.E. Consortium/LLNL at:
C    http://image.llnl.gov
C    Plate: LLAM10141  row: o  column: 24
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XX
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Homo sapiens mRNA; cDNA DKFZp566A093 (from clone DKFZp566A093); complete cds

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DE   Homo sapiens mRNA for Id-1H, complete cds.

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Homo sapiens mRNA for KIAA1254 protein, partial cds.

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)E Homo sapiens cDNA clone:HEMBA1001328, 3' end, expressed in whole embryo,
)E mainly head.

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Homo sapiens mRNA; cDNA DKFZp564F1862 (from clone DKFZp564F1862); complete cds

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DE Homo sapiens annexin A1, mRNA (cDNA clone MGC:5095 IMAGE:3459615), complete
DE cds.

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DE Homo sapiens peroxisomal D3,D2-enoyl-CoA isomerase, mRNA (cDNA clone MGC:3558 IMAGE:3608151), complete cds.

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DE   (cDNA clone MGC:50513 IMAGE:5742016), complete cds.

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Homo sapiens RTN2-A (RTN2) mRNA, complete cds.

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DE Human mRNA for KIAA0188 gene, partial cds.

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'E Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble),
'E mRNA (cDNA clone IMAGE:2819708), partial cds.

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T CYGGTAAVFNAVNWIESSSWDGRYALVVAGDIAVYATGNARPTGGVGAVALLIGPNAPL
T IFERGLRGTHMQHAYDFYKPDMLSEYPIVDGKLSIQCYLSALDRCYSVYCKKIHAQWQK
T EGNDKDFTLNDFGFMIFHSPYCKLVQKSLARMLLNDFLNDQNRDKNSIYSGLEAFGDVK
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T LAGKRIGVFSYGSGLAATLYSLKVTQDATPGSALDKITASLCDLKSRLDSRTGVAPDVF
T AENMKLREDTHHLVNYIPQGSIDSLFEGTWYLVRVDEKHRRTYARRPTPNDDTLDEGVG
T LVHSNIATEHIPSPAKKVPRLPATAAEPEAAVISNGEH"

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/

Homo sapiens S100 calcium binding protein A14, mRNA (cDNA clone MGC:11012 IMAGE:3640899), complete cds.

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aaaaaaaaa                                                           489
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'E Homo sapiens cDNA clone:ADBALE09, 5'end, expressed in human adrenal gland.
X

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/

as43b01.x1 Barstead aorta HPLRB6 Homo sapiens cDNA clone IMAGE:2319913 3', mRNA sequence.

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)E Homo sapiens drebrin 1, transcript variant 1, mRNA (cDNA clone MGC:1517
)E IMAGE:3356428), complete cds.

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'T VAEFFQGVDVIVNASSVEDIDAGAIGQRLSNGLARLSSPVLHRLRLREDENAEPVGTTY
'T QKTDAAVEMKRINREQFWEQAKKEEELRKEEERKKALDERLRFEQERMEQERQEQEERE
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DE Homo sapiens potentially prenylated protein tyrosine phosphatase hPRL-3
DE mRNA, complete cds.

FT /translation="MARMNRPAPVEVSYKHMRFLITHNPTNATLSTFIEDLKKYGATTV
FT VRVCEVTYDKTPLEKDGITVVDWPFDDGAPPPGKVVEDWLSLVKAKFCEAPGSCVAVHC
FT VAGLGRAPVLVALALIESGMKYEDAIQFIRQKRRGRINSKQLTYLEKYRPKQRLRFKDP
FT HTHKTRCCVM"

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Homo sapiens cell cycle progression restoration 8 protein (CPR8) mRNA, complete cds.

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)E Human channel-like integral membrane protein (CHIP28) mRNA, complete cds.
(X
(W channel-like integral membrane protein.

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Homo sapiens STRA6 isoform 1 mRNA, complete cds, alternatively spliced.

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Homo sapiens solute carrier family 7 (cationic amino acid transporter, y+ system), member 7, mRNA (cDNA clone MGC:1534 IMAGE:3504357), complete cds.

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)E 601440558F1 NIH_MGC_72 Homo sapiens cDNA clone IMAGE:3925214 5', mRNA
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DE Human DNA for insulin-like growth factor II (IGF-2); exon 7 and additional ORF.

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nac79g07.x1 NCI_CGAP_Brn23 Homo sapiens cDNA clone IMAGE:3440820 3', mRNA sequence.

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DE   Homo sapiens hypothetical protein MGC11256, mRNA (cDNA clone MGC:60219
DE   IMAGE:6091291), complete cds.

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```

Homo sapiens cDNA clone IMAGE:3952627, partial cds.

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)E Homo sapiens cDNA clone IMAGE:3952627, partial cds.

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PT1.1_07_C06.r tumor1 Homo sapiens cDNA 5', mRNA sequence.

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)E Homo sapiens cDNA FLJ12940 fis, clone NT2RP2005038, weakly similar to DNA
)E NUCLEOTIDYLEXOTRANSFERASE (EC 2.7.7.31).

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np60h03.s1 NCI_CGAP_Br2 Homo sapiens cDNA clone IMAGE:1130741 3', mRNA sequence.

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DE Homo sapiens ALL1-fused gene from chromosome 1q, mRNA (cDNA clone
DE IMAGE:2823316).

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Human mRNA for acetyl-coenzyme A transporter, complete cds.

acetyl-coenzyme A transporter.

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Homo sapiens SDF2L1 mRNA for SDF2 like protein 1, complete cds.

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ϽE Homo sapiens RTN2-A (RTN2) mRNA, complete cds.

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Homo sapiens cDNA: FLJ22209 fis, clone HRC01496.

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DE Homo sapiens UDP-N-acetylglucosamine-2-epimerase mRNA, complete cds.

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Homo sapiens carcinoembryonic antigen 2a (CGM2) mRNA, complete cds.

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Homo sapiens immediate early response 3, transcript variant short, mRNA (cDNA clone MGC:5118 IMAGE:3457670), complete cds.

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human full-length cDNA 3-PRIME end of clone CS0DA009YG15 of NEUROBLASTOMA of Homo sapiens (human)

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/

Homo sapiens organic anion transporter polypeptide-related protein 1 (OATPRP1) mRNA, complete cds.

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Homo sapiens cDNA: FLJ21243 fis, clone COL01164.

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)E ab38f03.s1 Stratagene HeLa cell s3 937216 Homo sapiens cDNA clone
)E IMAGE:843101 3' similar to contains Alu repetitive element;, mRNA sequence.

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/

Homo sapiens KPL1 (KPL1) mRNA, complete cds.

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Homo sapiens carboxypeptidase, vitellogenic-like, transcript variant 2, mRNA (cDNA clone MGC:10029 IMAGE:3888647), complete cds.

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Homo sapiens teratocarcinoma-derived growth factor 1, mRNA (cDNA clone MGC:24110 IMAGE:4615416), complete cds.

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'E   Homo sapiens lipase mRNA, complete cds.

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Homo sapiens v-fos FBJ murine osteosarcoma viral oncogene homolog, mRNA (cDNA clone MGC:11074 IMAGE:3688670), complete cds.

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)E complete cds.

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Human 150 kDa oxygen-regulated protein ORP150 mRNA, complete cds.

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Homo sapiens s-CaBP1 (CABP1) mRNA, complete cds.

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E Homo sapiens cDNA FLJ12397 fis, clone MAMMA1002769, weakly similar to Homo
E sapiens cell cycle progression restoration 8 protein (CPR8) mRNA.

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hn58g08.x1 NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:3032126 3', mRNA sequence.

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DE   Homo sapiens cDNA FLJ13465 fis, clone PLACE1003493, weakly similar to
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)E Homo sapiens heat shock 27kDa protein 1, mRNA (cDNA clone MGC:8509
)E IMAGE:2822325), complete cds.

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Homo sapiens carcinoembryonic antigen (CGM2) mRNA, complete cds.

carcinoembryonic antigen.

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DE   Homo sapiens keratin 7, mRNA (cDNA clone MGC:3625 IMAGE:3610347), complete
DE   cds.

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Homo sapiens hxCT mRNA for cystine/glutamate exchanger, complete cds.

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DE   clone MGC:8362 IMAGE:2819899), complete cds.

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     caaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a                          1781
/
```

Homo sapiens cDNA clone:HEMBA1000726, 3' end, expressed in whole embryo, mainly head.

```
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```
)E   Homo sapiens MDG1 mRNA, complete cds.
CX
CW   HTC.

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'T                       Q"

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Homo sapiens prostate stem cell antigen (PSCA) mRNA, complete cds.

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     tttctagctg tcaaaaaaaa aaa                                              1103
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E Homo sapiens interleukin 11 receptor, alpha, transcript variant 1, mRNA
E (cDNA clone MGC:2146 IMAGE:3502059), complete cds.

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T VTLQLGYPPARPVVSCQAADYENFSCTWSPSQISGLPTRYLTSYRKKTVLGADSQRRSP
T STGPWPCPQDPLGAARCVVHGAEFWSQYRINVTEVNPLGASTRLLDVSLQSILRPDPPQ
T GLRVESVPGYPRRLRASWTYPASWPCQPHFLLKFRLQYRPAQHPAWSTVEPAGLEEVIT
T DAVAGLPHAVRVSARDFLDAGTWSTWSPEAWGTPSTGTIPKEIPAWGQLHTQPEVEPQV
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DE   Homo sapiens mRNA; cDNA DKFZp564O2071 (from clone DKFZp564O2071); complete
DE   cds

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DE Homo sapiens collagen alpha 3 type IX (COL9A3) mRNA, complete cds.

alpha-3 type IX collagen; COL9A3 gene; collagen.

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Homo sapiens cDNA FLJ20113 fis, clone COL05437.

fis (full insert sequence); oligo capping.

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Homo sapiens calcium binding protein 1 (calbrain), mRNA (cDNA clone

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Homo sapiens negative growth-regulatory protein MyD118 (MYD118) mRNA, complete cds.

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Homo sapiens synaptogyrin 3, mRNA (cDNA clone MGC:20003 IMAGE:4334996), complete cds.

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Homo sapiens monocarboxylate transporter 2 (MCT2) mRNA, complete cds.

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'X
'W gonadotropin-releasing hormone receptor.

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Homo sapiens midline 1 (MID1) mRNA, complete cds.

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DE   Homo sapiens midline 1 (MID1) mRNA, complete cds.

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DE   Homo sapiens IL-1 receptor accessory protein mRNA, complete cds.

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Homo sapiens clone FLB0708 mRNA sequence.

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gtggctcacg cctgtaatcc cagcactttg ggaggccgag acgagcgaat cacgaggtca     180
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agaatggcat gaacccggga ggcggagctt gcagtgagcc gagatcgcgc cactgcactc     360
cagcctgggc gacagagcga gactccgtct gaaaaaaaaa aaaagggaaa aatcctgcca     420
catgatatgg tatgggtcaa acttgaagac attaagctaa ataaaatgtc agtcacaaaa     480
agacaaatat tatatgattc cactcacatg aagtatcaag taatcaaact cacagaaaaa     540
gaaagtaaaa ttgtggttgc caatggttca gggtgaaaaa aagggagtta gtgtttaatg     600
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tcacctgagc tcaggagttc gagactagcc tggccaaaat ggcataaccc catctctaca    1080
taaaatacaa aaatcagctg ggaacactgg tgcacacctg tagtctcagc tactcaggag    1140
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ccattgcact ccagcctggg tgacagagac agactctgct tggtcacttt tttggcggag    1260
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gagagcgaac agcggtgagt caggagcagg agcgtgcgga ccaaaaatcc tcagccctta    1800
cgaccgcgtc ttcctcaaaa aaaaa                                          1825
```

TABLE 2

	B+ Signal	Det.	Det. p-valu	B+ vs G+ SLR	Change	Change p-	[redacted]
202825_at	116.6	A	0.129639	-1	D	0.999853	
205844_at	188.6	P	0.001953	-1	D	0.99998	
204808_s_	134.5	P	0.018555	-1	D	0.999226	
205264_at	151	M	0.056152	-1	D	0.999308	
202687_s_	100.1	P	0.000244	-1	D	0.99998	
208323_s_	2738.1	P	0.000244	-1	D	0.99998	
206239_s_	585.3	P	0.000244	-1	D	0.99998	
207655_s_	98.9	P	0.018555	-1	D	0.99997	
220041_at	162.8	P	0.030273	-1	D	0.99998	
203178_at	26.8	P	0.030273	-1	D	0.99987	
218747_s_	18.1	A	0.303711	-1	D	0.999611	
217933_s_	482	P	0.001953	-1	D	0.99998	
214373_at	110.7	A	0.111572	-1	D	0.999693	
205552_s_	283.7	P	0.000244	-1	D	0.99998	
211172_x_	53.7	A	0.067627	-1	D	0.998923	
204228_at	150.5	A	0.095215	-1	D	0.998923	
203787_at	41.5	P	0.010742	-1	D	0.996301	
204994_at	174.3	P	0.00415	-1	D	0.998664	
203567_s_	106.2	A	0.129639	-1	D	0.999811	
215464_s_	92.4	M	0.056152	-1.1	D	0.998923	
218280_x_	275.2	P	0.000732	-1.1	D	0.99998	
AFFX-HUI	177.9	P	0.012547	-1.1	D	1	
219211_at	64.8	A	0.303711	-1.1	D	0.999886	
219691_at	83.8	P	0.000244	-1.1	D	0.99998	
217761_at	479	P	0.000732	-1.1	D	0.99998	
214022_s_	1101.3	P	0.000244	-1.1	D	0.99997	
218017_s_	48	A	0.27417	-1.1	D	0.99987	
214290_s_	547.9	P	0.000244	-1.1	D	0.99998	
216565_x_	179.8	P	0.010742	-1.2	D	0.999973	
204739_at	45.3	A	0.080566	-1.2	D	0.999759	
AFFX-HUI	399.3	P	0.000225	-1.2	D	1	
200790_at	481.6	P	0.001953	-1.2	D	0.99998	
202446_s_	982.6	P	0.000244	-1.2	D	0.99998	
203903_s_	182.8	P	0.000732	-1.2	D	0.99998	
AFFX-HUI	35.8	A	0.313723	-1.2	D	0.99985	
219366_at	126.2	A	0.080566	-1.2	D	0.999135	
206332_s_	118.9	P	0.000732	-1.2	D	0.99996	
202269_x_	29.2	A	0.171387	-1.2	D	0.99775	
201601_x_	664.8	P	0.000244	-1.2	D	0.99998	
AFFX-HUI	126.9	P	0.000081	-1.2	D	1	
202430_s_	281.8	P	0.000244	-1.2	D	0.99994	
208268_at	13.3	A	0.366211	-1.3	D	0.996959	
202388_at	474.9	P	0.001953	-1.3	D	0.99998	
204259_at	445.8	P	0.037598	-1.3	D	0.999833	
220084_at	56.4	P	0.010742	-1.3	D	0.99996	
200887_s_	583.7	P	0.000244	-1.3	D	0.99998	
218943_s_	67.2	A	0.27417	-1.3	D	0.999899	
219209_at	114.9	P	0.00293	-1.4	D	0.999954	
209969_s_	95.1	P	0.037598	-1.4	D	0.99998	
208965_s_	43.9	A	0.111572	-1.4	D	0.999654	
215252_at	41.2	A	0.334473	-1.5	D	0.99751	
208966_x_	98	P	0.001221	-1.5	D	0.99996	
203372_s_	13	A	0.129639	-1.5	D	0.995927	

AFFX-HUI	61.3	P	0.004998	-1.5	D	1	[redacted]
210738_s_	13.8	A	0.129639	-1.6	D	0.997968	[redacted]
210163_at	9.2	M	0.056152	-1.6	D	0.999973	[redacted]
215447_at	15.1	A	0.432373	-1.6	D	0.996645	[redacted]
219352_at	93.9	P	0.046143	-1.7	D	0.99998	[redacted]
203908_at	71.5	P	0.001953	-1.7	D	0.999932	[redacted]
205345_at	23.8	A	0.366211	-1.7	D	0.999954	[redacted]
AFFX-r2-I	74.9	A	0.129639	-1.7	D	0.999973	[redacted]
203153_at	341.9	P	0.008057	-1.8	D	0.99998	[redacted]
213797_at	69.3	A	0.219482	-1.8	D	0.999727	[redacted]
206664_at	54.5	P	0.001953	-1.8	D	0.99998	[redacted]
202086_at	167.8	P	0.010742	-1.8	D	0.99998	[redacted]
216200_at	4	A	0.533936	-1.8	D	0.999654	[redacted]
214059_at	78.5	P	0.000244	-1.9	D	0.99998	[redacted]
205771_s_	225.8	P	0.00415	-1.9	D	0.99998	[redacted]
204972_at	190.4	P	0.00293	-1.9	D	0.99998	[redacted]
218986_s_	75.5	P	0.01416	-1.9	D	0.999922	[redacted]
207057_at	9.2	A	0.432373	-1.9	D	0.995927	[redacted]
214453_s_	155.9	P	0.001221	-2.2	D	0.99998	[redacted]
215729_s_	21.2	P	0.01416	-2.4	D	0.999973	[redacted]
211520_s_	4.2	A	0.72583	-2.4	D	0.997247	[redacted]
213293_s_	83.8	P	0.023926	-2.5	D	0.999693	[redacted]
204439_at	110.5	P	0.018555	-2.8	D	0.99998	[redacted]
202664_at	1.8	A	0.432373	-2.8	D	0.999611	[redacted]
215241_at	13.6	A	0.432373	-3	D	0.999382	[redacted]
204615_x_	1342.2	P	0.001953	1	I	0.000027	[redacted]
205128_x_	548.8	P	0.001221	1	I	0.000027	[redacted]
221760_at	598.4	P	0.000244	1	I	0.00002	[redacted]
204044_at	250.1	P	0.01416	1	I	0.000273	[redacted]
205939_at	125	P	0.000244	1	I	0.000023	[redacted]
201749_at	265.7	P	0.000732	1	I	0.000101	[redacted]
201626_at	1377.8	P	0.000244	1	I	0.00002	[redacted]
31637_s_a	882	P	0.007543	1	I	0.000271	[redacted]
201627_s_	2066.8	P	0.000244	1	I	0.000068	[redacted]
213348_at	207.5	P	0.001953	1	I	0.000167	[redacted]
213154_s_	153.2	P	0.005859	1	I	0.00002	[redacted]
45714_at	259.6	P	0.007543	1	I	0.000008	[redacted]
200599_s_	2866.6	P	0.000244	1	I	0.00002	[redacted]
203252_at	323.6	P	0.000732	1	I	0.000027	[redacted]
214581_x_	415.5	P	0.000244	1	I	0.000046	[redacted]
203207_s_	296.6	P	0.000244	1	I	0.00002	[redacted]
217168_s_	2143.1	P	0.000244	1	I	0.00002	[redacted]
218627_at	204.5	P	0.00415	1	I	0.003041	[redacted]
218145_at	2828.4	P	0.000244	1	I	0.00002	[redacted]
200598_s_	1610.6	P	0.000244	1	I	0.00002	[redacted]
220892_s_	1246.1	P	0.000244	1	I	0.000023	[redacted]
212274_at	227.2	P	0.037598	1	I	0.000046	[redacted]
213448_at	125.3	P	0.023926	1	I	0.001486	[redacted]
40093_at	378.7	P	0.001354	1	I	0.000012	[redacted]
212272_at	95.6	P	0.037598	1	I	0.000389	[redacted]
205830_at	97.3	P	0.00293	1	I	0.001077	[redacted]
212218_s_	1346.5	P	0.000244	1	I	0.00002	[redacted]
201005_at	1763.2	P	0.000244	1	I	0.00002	[redacted]
214152_at	210	P	0.005859	1	I	0.000023	[redacted]
204058_at	230	P	0.000244	1	I	0.000068	[redacted]
201790_s_	837.4	P	0.000244	1	I	0.00002	[redacted]

209218_at	3285	P	0.000244	1	I	0.00002	[redacted]
218963_s_	116	P	0.046143	1.1	I	0.00225	[redacted]
217790_s_	66.7	P	0.023926	1.1	I	0.00225	[redacted]
204059_s_	376.1	P	0.000732	1.1	I	0.000023	[redacted]
220451_s_	123.5	P	0.008057	1.1	I	0.001486	[redacted]
217025_s_	116.4	P	0.005859	1.1	I	0.000027	[redacted]
204205_at	73.9	P	0.00293	1.1	I	0.000189	[redacted]
210069_at	71.8	P	0.008057	1.1	I	0.001651	[redacted]
208116_s_	296.2	P	0.000244	1.1	I	0.00002	[redacted]
221577_x_	780	P	0.000244	1.1	I	0.00002	[redacted]
210202_s_	141.7	P	0.00415	1.1	I	0.003699	[redacted]
212119_at	419.1	P	0.008057	1.1	I	0.000035	[redacted]
203875_at	76.3	P	0.00293	1.1	I	0.001336	[redacted]
214315_x_	860.5	P	0.000244	1.1	I	0.00002	[redacted]
213802_at	50.6	P	0.01416	1.1	I	0.000774	[redacted]
213424_at	36.8	P	0.001221	1.1	I	0.000865	[redacted]
203675_at	165.5	P	0.000244	1.1	I	0.00006	[redacted]
202275_at	506.7	P	0.001221	1.1	I	0.00003	[redacted]
206683_at	116.8	P	0.001953	1.1	I	0.00002	[redacted]
221750_at	428.6	P	0.000244	1.2	I	0.00002	[redacted]
205127_at	77.7	P	0.037598	1.2	I	0.000023	[redacted]
208291_s_	250.4	P	0.001953	1.2	I	0.000438	[redacted]
221485_at	888.9	P	0.000244	1.2	I	0.00002	[redacted]
208763_s_	717.7	P	0.00415	1.2	I	0.00002	[redacted]
208937_s_	1120.4	P	0.000244	1.2	I	0.00002	[redacted]
221511_x_	569.9	P	0.001953	1.2	I	0.00002	[redacted]
214151_s_	245.6	P	0.018555	1.2	I	0.00002	[redacted]
209850_s_	342.9	P	0.046143	1.2	I	0.00003	[redacted]
202842_s_	1130.8	P	0.000244	1.2	I	0.00002	[redacted]
201012_at	965.6	P	0.000244	1.2	I	0.00002	[redacted]
218025_s_	85.8	P	0.00293	1.2	I	0.000438	[redacted]
206125_s_	270.5	P	0.030273	1.2	I	0.000438	[redacted]
204217_s_	217.4	P	0.010742	1.2	I	0.000035	[redacted]
212276_at	299	P	0.000244	1.2	I	0.000167	[redacted]
205822_s_	405.7	P	0.000244	1.2	I	0.00002	[redacted]
218677_at	815.4	P	0.000244	1.2	I	0.00002	[redacted]
209146_at	1219.4	P	0.000244	1.2	I	0.00002	[redacted]
202557_at	157.3	P	0.000244	1.2	I	0.00003	[redacted]
202806_at	148.1	P	0.008057	1.2	I	0.000189	[redacted]
206574_s_	181.3	P	0.00415	1.2	I	0.000241	[redacted]
221156_x_	241.4	P	0.000732	1.3	I	0.000023	[redacted]
209047_at	237.1	P	0.001953	1.3	I	0.00003	[redacted]
221701_s_	403.1	P	0.010742	1.3	I	0.000046	[redacted]
204588_s_	468.7	P	0.00415	1.3	I	0.00002	[redacted]
212120_at	565.7	P	0.000244	1.3	I	0.00002	[redacted]
202409_at	506.9	P	0.000244	1.3	I	0.00002	[redacted]
213716_s_	438.8	P	0.008057	1.3	I	0.000114	[redacted]
218358_at	810.5	P	0.00293	1.3	I	0.000023	[redacted]
211031_s_	252.7	P	0.00415	1.3	I	0.00002	[redacted]
47560_at	343	P	0.003067	1.3	I	0.000191	[redacted]
222238_s_	103.3	P	0.037598	1.3	I	0.000389	[redacted]
213577_at	802.1	P	0.000244	1.3	I	0.000023	[redacted]
211071_s_	237.8	P	0.00293	1.3	I	0.00004	[redacted]
208608_s_	864.2	P	0.000244	1.4	I	0.00002	[redacted]
203165_s_	142.1	P	0.000244	1.4	I	0.00002	[redacted]
218681_s_	364.1	P	0.001221	1.4	I	0.000023	[redacted]

34408_at	506.7	P	0.000219	1.4	I	0
216449_x_	733.5	P	0.000732	1.4	I	0.000035
205042_at	589.2	P	0.000244	1.4	I	0.000046
208121_s_	282.2	P	0.000244	1.4	I	0.00002
206199_at	2294.8	P	0.000244	1.4	I	0.00002
217594_at	24.6	P	0.046143	1.4	I	0.004481
201631_s_	1124.5	P	0.000244	1.5	I	0.00002
212345_s_	306.8	P	0.000244	1.5	I	0.00002
202539_s_	704.5	P	0.000732	1.5	I	0.000052
213562_s_	892.1	P	0.000244	1.5	I	0.00002
219911_s_	925.8	P	0.000244	1.5	I	0.00002
212944_at	767.8	P	0.000244	1.5	I	0.00002
217678_at	334.6	P	0.000244	1.5	I	0.000023
209504_s_	430.9	P	0.00293	1.5	I	0.000027
208146_s_	175.9	P	0.005859	1.6	I	0.000027
206286_s_	266	P	0.001953	1.6	I	0.000068
221679_s_	54	P	0.030273	1.6	I	0.002753
209189_at	256.1	P	0.008057	1.6	I	0.00002
211936_at	3417.5	P	0.000244	1.6	I	0.00002
204268_at	483.6	P	0.000732	1.6	I	0.00002
213164_at	945	P	0.000244	1.6	I	0.00002
200825_s_	1824.2	P	0.000244	1.6	I	0.00002
210181_s_	58.4	P	0.030273	1.6	I	0.000046
222156_x_	153.9	P	0.000244	1.7	I	0.00003
212122_at	67.4	P	0.00415	1.7	I	0.000241
219091_s_	379	P	0.000244	1.7	I	0.000027
201841_s_	1572.5	P	0.000244	1.7	I	0.00002
206198_s_	876.6	P	0.000244	1.7	I	0.00002
211848_s_	974.2	P	0.000244	1.7	I	0.00002
209016_s_	56.2	P	0.030273	1.8	I	0.000167
209921_at	458.5	P	0.000732	1.8	I	0.00002
204540_at	1656.5	P	0.000244	1.8	I	0.00003
215058_at	68.5	P	0.018555	1.8	I	0.00249
202843_at	185.7	P	0.001221	1.9	I	0.00002
205319_at	201.2	P	0.001953	1.9	I	0.000088
202655_at	700.7	P	0.001953	2	I	0.000027
204773_at	72.3	P	0.01416	2.2	I	0.004073
202887_s_	3008.2	P	0.000244	2.2	I	0.00002
204724_s_	292.3	P	0.010742	2.2	I	0.00002
201246_s_	106.5	P	0.000244	2.3	I	0.002032
208868_s_	60.7	P	0.010742	2.4	I	0.000618
209443_at	310.9	P	0.00293	2.5	I	0.000241
207761_s_	81.7	P	0.018555	2.5	I	0.000035
208321_s_	86.8	P	0.018555	2.5	I	0.003355
213201_s_	575.3	P	0.000244	3.1	I	0.00002
207574_s_	81.7	P	0.00293	3.1	I	0.000023
212702_s_	97.4	M	0.056152	3.3	I	0.000088
205691_at	114	M	0.056152	3.8	I	0.000273
201105_at	916.1	P	0.000244	4.8	I	0.00002
210807_s_	34.8	A	0.171387	-1.1	MD	0.994067
216341_s_	26	A	0.111572	-1.2	MD	0.994067
203637_s_	25.8	A	0.111572	-1.6	MD	0.994591
205227_at	34.9	A	0.129639	-1.8	MD	0.995075
216247_at	79.4	P	0.00293	1	MI	0.004925

[redacted]

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